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A MANUAL  
OF THE  
PARASITIC PROTOZOA OF MAN

BY  
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## PREFACE

THE great importance of protozoan parasites in human pathology is now well recognized. Parasites belonging to the PROTOZOA are responsible for some of the most important and serious infections of man, as the malarial fevers, amœbic dysentery, sleeping sickness, kala-azar, tropical ulcer, and Chagas' disease, and for this reason a work which contains all of the important facts that have been learned regarding the morphology, life-history, relation to disease, prophylaxis, and diagnosis of these parasites should prove useful, especially as much of this knowledge is available only in monographs devoted to special genera or species of protozoa.

At the present time there is no work in English that adequately covers this field, from a medical standpoint, and during an experience of almost thirty years in the study of the parasitic protozoa of man and related organisms, nearly ten years of which have been spent in teaching, the need of a complete manual devoted to this subject has been keenly felt many times by the writer. During this time material intended for such a work has been accumulated and the present volume is the result.

This manual is not a zoological treatise but is intended for the use of health officers, medical practitioners, teachers, laboratory and research workers, and medical students. It does, however, contain what is believed to be an adequate discussion of the history, nomenclature, and generic and specific position of each of the organisms described, from the viewpoint of the medical practitioner and student.

It is believed that this manual contains every fact of real importance that is known regarding the various parasites described, and, in many instances, it has been found essential to consider closely related forms in the lower animals, as well as free-living species, as the differential diagnosis of such species from the parasitic species of man is often most important. It has also been necessary, in some instances, to consider coprozoic organisms that may give rise to confusion in diagnosis, so that the manual, while devoted primarily to the parasitic protozoa of man, actually includes numerous other organisms living in the lower animals or free in nature.

Each organism has been considered under the following headings: Synonyms; History and Nomenclature; Morphology in Living and Stained Preparations; Resistance to Injurious Agencies; Habitat; Species Occurring in Lower Animals; Cultivation; Life-history; Method of Reproduction; Geographical Distribution; Incidence of Infection; Method of Transmission; Experimental Infection of Lower Animals; Rela-

tion to Disease; Pathology; Prophylaxis; and Diagnosis. Under each of these divisions there has been included, as briefly as possible, all of the data of importance that have been accumulated, either as the result of laboratory research or clinical experience, relating to each parasite described. Under the heading of "Diagnosis" all laboratory methods that have been found of value in the diagnosis of each organism have been described and a Technical Appendix has been added which contains the most useful methods of cultivating and staining the various parasitic protozoa.

It will be noted that a discussion of the *Spirochætes*, *Rickettsia*, and organisms usually classed in the CHLAMYDOZOA is omitted. This omission is intentional, as there is, at present, no evidence that any of these organisms are protozoan in nature beyond the personal opinions of those who desire them to be so classed, and there is a very considerable amount of evidence available that indicates that all of these organisms are much more closely allied to the BACTERIA than to the PROTOZOA. For this reason, it is not believed that their discussion should be included in a work devoted entirely to protozoan organisms.

For the convenience of the student a list of references is appended to each chapter. These lists should not be considered as a complete bibliography of the subject treated, as they contain only the contributions actually consulted in the preparation of the Manual, but they are sufficiently full to cover each of the subjects treated. An endeavor has been made to include in the consideration of each organism the most recent work that has been accomplished, and it will be noted that the reference lists are largely composed of comparatively recent contributions, as, in almost every subject discussed, the most valuable work is the most recent. In the text it will be noted that after the name quoted there is included in parentheses the date of the author's publication referred to in the text, and in order to use the reference list all that is necessary is to find in the list the name of the author followed by the same date given in the text. It has been the aim of the writer to give credit, in every instance, where it is due, and if there has been any omission in this respect it has been unintentional. It has not always been possible to agree with the opinions of certain authorities upon certain subjects, but, where such a disagreement is indicated in the text, it should be remembered that the opinion expressed is a personal one arrived at after a personal study of the subject and that, in a field where changes are constant as the result of added knowledge, it may well be that the writer is mistaken in his interpretation of the phenomena described. It has been the aim of the writer to avoid captious or caustic criticism of the work of others, and it is hoped that no injustice has been



done any worker in protozoology whose contributions have been referred to in the text.

The writer desires to express his indebtedness to the works of Dobell, Dobell and O'Connor, Wenyon and O'Connor, Kofoid and Swezy, Calkins, Stiles and Boeck, and Hegner and Taliaferro for much valuable data, and his thanks to Stiles and Boeck, and to Kofoid and his co-workers, for permission to reproduce numerous illustrations from their papers. His thanks are also due to Major-General Merritte W. Ireland, Surgeon General, U. S. Army, for permission to use official data and to reproduce the beautiful photomicrographs illustrating Bulletin No. 1, Surgeon General's Office, now out of print.

HONOLULU, T. H.

CHARLES F. CRAIG.

December 1, 1925.





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# PARASITIC PROTOZOA OF MAN

## CHAPTER I

THE PROTOZOA. CLASSIFICATION. STRUCTURE. PHYSIOLOGY.  
REPRODUCTION. PROTOZOA AS PARASITES. PARASITES  
AND PARASITISM.

The **Protozoa** are animals composed of a single cell. They form one of the two great divisions of the **ANIMAL KINGDOM**, the other being the **METAZOA**, or multicellular animals. While the **PROTOZOA** are usually defined as unicellular animals, Dobell (1911) and Dobell and O'Connor (1921) believe that this definition is misleading in that it compares the entire body of a protozoon with a single cell in the body of a metazoon, and that a better definition of the **PROTOZOA** would be that they are non-cellular animals. There is much to recommend this definition of the **PROTOZOA**, for while the animals in this group are unicellular in that their bodies do not contain groups of cells, the presence of various specialized parts, or organelles, as they are called, which perform special functions, makes them very different from our conception of a cell, as illustrated in metazoan animals, which forms only a minute portion of the body. In this work I shall follow the definition of the **PROTOZOA** as given by Dobell, *i.e.*, the division of the **ANIMAL KINGDOM** containing all non-cellular animals.

**Classification.**—The **PROTOZOA** are divided into four great groups, or **PHYLA**. These are the **RHIZOPODA** or **SARCODINA**, the **MASTIGOPHORA**, the **SPOROZOA**, and the **CILIOPHORA** or **INFUSORIA**. Each of these **PHYLA** contains multitudes of species, most of which are not parasitic but live free in nature and are known as “free-living” species. The **PHYLUM SPOROZOA** contains only parasitic species.

Each of the **PHYLA** contains animals that are important parasites of man. Some of these species cause serious disease in their human host, while others are apparently harmless, although living in man and dependent upon him for their existence.

**Phylum (Class) I.**—**RHIZOPODA** (**SARCODINA**). Protozoa that move by means of prolongations of the ectoplasm of the body, called pseudopodia. The protoplasm is divided into an outer portion, or ectoplasm, and an inner portion, or endoplasm, both being well differentiated. Contains parasitic and free-living species.

**Phylum II.**—**MASTIGOPHORA**. Protozoa that move by means of thread-like or whip-like filaments known as flagella. The protoplasm is

not as definitely divided into ectoplasm and endoplasm as in the RHIZOPODA. Contains both parasitic and free-living species.

**Phylum III.—SPOROZOA.** Protozoa that are not provided with any special organ of locomotion, but, when motile, usually depend upon contraction of the cytoplasm of the body for motility. All species are parasitic. Ectoplasm and endoplasm not differentiated distinctly in most species.

**Phylum IV.—CILIOPHORA (INFUSORIA).** Protozoa that move by means of thread-like, short filaments, known as cilia. Both parasitic and free-living species occur in this PHYLUM.

**Structure of the Protozoa.**—A protozoon is composed of a mass of *protoplasm* having a finely or coarsely granular appearance in the living condition. Typically, the protoplasm is divided into two portions, an outer, finely granular, hyaline portion called the *ectoplasm*, and an inner, more coarsely granular and less hyaline portion, called the *endoplasm*. Some of the PROTOZOA are covered by membranes or shell-like investments, but all of the species parasitic in man are devoid of such coverings except in the resting or cystic stage of development.

The *ectoplasm* fulfills the functions of movement, respiration, secretion, ingestion of food, excretion, and protection, while the *endoplasm* performs the function of digestion and contains the structures concerned in reproduction.

The *ectoplasm* gives rise to the structures concerned in motility, which are called *ectoplasmic organelles*. These consist in the RHIZOPODA of prolongations of the ectoplasm called *pseudopodia*; in the MASTIGOPHORA of long lash-like filaments called *flagella*; and in the CILIOPHORA of spine-like flexible structures called *cilia*. Not only are the organelles mentioned concerned in motility, but they also assist in the capture of food and probably have much to do with sensation. Besides the organelles mentioned the ectoplasm of some of the PROTOZOA contains contractile bands called *myonemes*, which also assist in locomotion.

*Contractile vacuoles* are present in certain protozoan organisms, the function of which is to regulate the osmotic pressure by the elimination of water or to eliminate waste materials. These vacuoles originate in the ectoplasm, in the opinion of most protozoologists, but they are apparently contained within the endoplasm.

In certain species of PROTOZOA the ectoplasm contains a definite opening for the ingestion of food, called the *cytostome*, and this may have a well-defined tube leading into it, called the *cytopharynx*, through which the food passes and eventually enters the endoplasm.

The ectoplasm also acts as a protective covering for the endoplasm and in some species is composed of membranous or shell-like substances.

The *endoplasm* contains certain structures that are known as *endo-*



*plasmic organelles* and which preside over nutrition and reproduction. These consist of the *nucleus* and *food vacuoles*.

The protozoan *nucleus* is of great interest to the protozoologist as the classification of some of the PROTOZOA into species, and even into genera, is based upon the structure of this portion of the endoplasm.

The *structure* of the *nucleus* varies greatly in different genera and species. In some it is simply a mass of chromatin without definite structure, while in others it has a very definite and complex structure. In species in which the nucleus is most highly differentiated as regards structural details, the following structures may be distinguished: the nuclear membrane, the karyosome, the linin net-work, chromatin granules, and the centrosome, which may be situated within the karyosome or outside of the nucleus.

The *nuclear membrane* constitutes the covering of the nucleus, separating it from the endoplasm. It may be visible as a thick or thin, definite membrane, circular in outline, or it may be invisible as a definite membrane. It is composed of achromatinic substances but may be lined internally with *chromatin granules*.

The *karyosome* is a small, generally rounded mass lying within the nuclear cavity, usually at or near the centre of the nucleus. It may be composed of a mixture of plastin and chromatin or of chromatin alone.

The *linin net-work* is a net-work of delicate fibrils lying in the space between the nuclear membrane and the karyosome. It stains poorly and is not visible in some nuclei.

The *chromatin granules* may be composed of plastin and chromatin or of chromatin alone, and may line the nuclear membrane or occur at the nodal points of the linin net-work or both. When the linin net-work is invisible the chromatin granules or masses may be apparently free in the space between the karyosome and the nuclear membrane.

The *centrosome* is a minute granule which may lie within the karyosome or entirely outside of the nucleus. When thus located it is generally in close association with the point of origin of the organelles of motility and is called a *blepharoplast*.

In some of the PROTOZOA there are two or more nuclei, one called the *vegetative* or *macronucleus* and the other the *generative* or *miconucleus*.

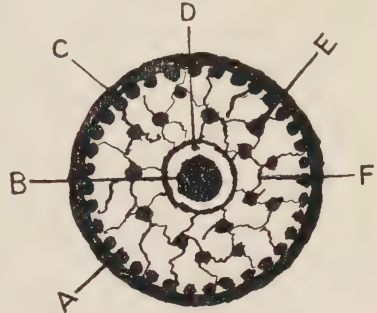


FIG. 1.—Schematic diagram of the nucleus of a protozoan, showing minute structure as illustrated especially in the amœbæ. A. Nuclear membrane. B. Karyosome. C. Chromatin granules lining the interior of the nuclear membrane. D. Unstained space, or halo, surrounding the karyosome. E. Chromatin granules in space between nuclear membrane and karyosome, lying at the intersections of the linin net-work. F. Linin net-work.

Besides the nucleus the endoplasm contains *chromidia*, *food vacuoles*, and various *ingested substances*, as crystals, bacteria, and vegetable cells.

*Chromidia* occur in the endoplasm of many of the PROTOZOA and are composed of chromatin which is probably of nuclear origin. The chromidia may be scattered throughout the endoplasm or collected in more or less irregular masses in certain portions of the endoplasm. The exact function of the chromidia is not known.

*Food vacuoles* may be numerous and scattered throughout the endoplasm or only a few may be present. They may contain ingested material or appear simply as vacuoles within the endoplasm.

*Ingested material* may be present in the endoplasm and the character of such material differs in different genera and species. Vegetable cells, crystals, and bacteria are most frequently observed within the food vacuoles, or in the endoplasm, but other protozoan organisms, leucocytes, and even red blood corpuscles may occur in some of the PROTOZOA. A notable example is *Endamoeba histolytica*, the pathogenic parasitic amœba of man, which ingests the red blood corpuscles of its host in large numbers.

In some of the free-living species a contractile vacuole is present, and always in the free-living amœbæ.

**Physiology of the Protozoa.**—The physiological processes of the PROTOZOA are of special interest because all of them are performed in a body composed of a single cell. In order to perform the various physiological processes necessary for the life of these organisms certain organelles have been developed, some concerned in motility and sensation, others in the procuring and digestion of food, and still others in respiration, secretion, excretion, and reproduction.

1. **Respiration.**—In the PROTOZOA respiration may be of the aerobic or anaerobic type. The free-living protozoa absorb oxygen through the ectoplasm and eliminate carbon dioxide in the same manner. Others live under conditions in which oxygen is absent or very limited in amount, and in such respiration is accomplished anaerobically by the splitting up of complex chemical substances into simple compounds.

2. **Nutrition.**—The method of capturing food and digesting and eliminating it varies greatly in different protozoa. In the RHIZOPODA (SARCODINA) most of the organisms capture their food by means of prolongations of the ectoplasm called pseudopodia, ingest the food by covering it with the protoplasm, and transfer it to food vacuoles in the endoplasm, where it is digested by ferments secreted by the organism. The nutritive materials are absorbed by the endoplasm and the waste materials are eliminated. When contractile vacuoles are present, it is believed by some authorities that these act as excretory organelles for waste materials.

In the MASTIGOPHORA food is captured by means of flagella or pseudopodia, or nutritive material is absorbed from the body fluids or tissues in which the organisms are living as parasites. Many of the MASTIGOPHORA are holophytic or saprophytic as regards nutrition, but the holozoic type of nutrition is also common in this PHYLUM. In the protozoan parasites of the blood the type of nutrition is saprophytic.

In the most highly developed of the MASTIGOPHORA the ingested food reaches the interior of the organism through a mouth or cytostome. Digestion is accomplished by means of ferments secreted by the organisms.

In the SPOROZOA, which are all parasitic in nature, nutrition is accomplished entirely by absorption of food material from the fluids or tissues of the host, as these organisms have no pseudopodia, flagella, or cilia, and are devoid of a mouth or food vacuoles.

In the CILIOPHORA (INFUSORIA) nutrition is holozoic in type, except in rare instances, and the capture of food is generally accomplished by the movements of the cilia, which are very highly differentiated for this purpose in many species. The cilia guide the food into a mouth, or cytostome, provided with a cytopharynx terminating in the endoplasm. The food passes through the cytopharynx and when it reaches the endoplasm a food vacuole is formed in which it is digested by ferments secreted by the endoplasm. Nutritive materials are absorbed by the endoplasm and waste materials are excreted through a definite opening called the anal pore. In some species the capture of food is accomplished directly by the mouth, as cilia differentiated for that purpose are not present.

3. **Secretion.**—As already stated, the PROTOZOA secrete ferments which serve to digest the food materials necessary for nutrition. In addition, various other substances are secreted by the PROTOZOA, as enzymes, toxins, pigments, and gases. Some of the RHIZOPODA secrete toxic materials that kill or inhibit the motility of other organisms that serve as food and *Endamæba histolytica*, the cause of amœbic dysentery, secretes a proteolytic enzyme that destroys the tissue cells of its host. Whether this parasite secretes other toxins is not known, but some authorities believe that some of the acute symptoms of amœbic dysentery are due to toxins secreted by the amœbæ.

In the MASTIGOPHORA digestive ferments, enzymes, and pigments are secreted. Some authorities believe that toxins are secreted by the intestinal flagellates which are injurious to the host, but this has not been proven. Trypanotoxins have apparently been proven to be secreted by pathogenic trypanosomes, and it is believed by many that toxins are secreted by *Leishmania donovani*, the cause of kala-azar, although such toxins have not been isolated.

The SPOROZOA undoubtedly secrete digestive ferments and, as all of these organisms are parasitic in nature, it is probable that they also secrete toxic substances which act directly upon the tissues in which they live. The malaria plasmodia undoubtedly secrete a toxin which acts upon the red blood corpuscles in which they develop, as well as toxins which produce the general symptoms of a malarial paroxysm.

The CILIOPHORA (INFUSORIA) secrete ferments, enzymes, and, in the case of *Balantidium coli*, a proteolytic enzyme and a hæmolysin.

4. **Excretion.**—In the RHIZOPODA (SARCODINA) excretion of waste materials is accomplished by diffusion through the body surface, by precipitation, or by means of a contractile vacuole. The opinion of authorities differs regarding the function of the contractile vacuole, but there would appear to be little doubt that it assists in the excretion of waste material by removing excess fluid from the body, and in this manner acts as an excretory organelle.

In the MASTIGOPHORA excretion is accomplished by diffusion through the body surface.

In the SPOROZOA diffusion and precipitation are the methods of excretion that have been studied. In the malaria plasmodia the pigment formed by the action of the parasites upon the red blood corpuscles is a precipitation product and is excreted by the breaking up of the plasmodia into segments, or spores, at the time of sporulation, when the pigment is liberated into the blood serum.

In the CILIOPHORA (INFUSORIA) excretion of waste products takes place through a differentiated opening on the surface of the body known as the anal pore.

5. **Motility.**—As already stated, motility in the PROTOZOA is accomplished by means of pseudopodia, flagella, cilia, undulating membranes, and by contractions of the body, and the character of the motility varies with the organelles which produce it, so that many forms of motility are illustrated in these animals.

In the RHIZOPODA (SARCODINA) motility is accomplished by means of pseudopodia formed by the ectoplasm into which the endoplasm flows, thus producing a characteristic form of motion known as amœboid motion. The pseudopodia in the amœbæ of interest in human pathology may be lobose, spinose, or finger-like in shape, and may be protruded slowly or rapidly from the ectoplasm. The exact nature of amœboid motion is still undetermined, but it is now thought to be due to changes in the colloids in the protoplasm of the amœbæ.

In the MASTIGOPHORA motility is generally accomplished by means of flagella and, in some species, by undulating membranes. Limited amœboid motility also occurs in many members of this PHYLUM. The flagella



may be tractile or propulsive in nature and in many of the flagellates parasitic in man, both types of flagella occur.

In the SPOROZOA a very limited type of amœboid motility occurs in some species, but none of the organisms in this PHYLUM are provided with definite organelles of locomotion. The type of amœboid motility that may be present in some species is well illustrated in the young trophozoites of the malaria plasmodia, in which pseudopodia are protruded more or less rapidly and the parasites may change their position slightly in the infected red blood corpuscles by the aid of these organelles. In the development of the plasmodia in the stomach of the mosquito the zygote possesses a vermicular motility which enables it to penetrate the epithelial lining of the insect's stomach. The sporozoites are also endowed with motility, which enables them to finally reach the salivary glands and ducts of the mosquito.

In the CILIOPHORA (INFUSORIA) motility is accomplished by means of the cilia which cover the external surface of these animals, movement being produced by the rhythmical beating of the rows of cilia. Movement may be very rapid or slow and may be directly progressive or jerky in character.

6. **Reproduction.**—In the PROTOZOA reproduction may be *asexual* or *sexual* in character. If *asexual*, reproduction occurs by simple fission, in which the organism divides into two organisms, or by multiple fission, in which several organisms are produced by the parent organism. The division of the body of the protozoon is always preceded by the division of the nucleus or nuclei, and nuclear division may be either *mitotic* or *amitotic* in type. If mitotic in character, which is most frequently the type observed in the PROTOZOA, the nuclear changes are similar to those observed during mitotic division in metazoan cells. If amitotic, the chromatin of the nucleus divides directly into two portions, followed by the binary division of the body of the organism. In many of the PROTOZOA it has been impossible to determine whether nuclear division is mitotic or amitotic, and in many this process partakes of the characteristics of both types of nuclear division.

*Reproduction within a cyst* is frequently observed in the PROTOZOA, the organisms secreting resistant cystic walls within which division occurs by simple or multiple division. In many species, however, encystment is purely a protective process only occurring when conditions favorable for vegetative life have ceased, and in such organisms no reproduction occurs within the cysts. In some species encystment is preceded by conjugation or is the result of the fertilization of macrogametes by microgametes.

*Sexual* reproduction is common in the PROTOZOA and is generally associated with an alternation of generations and life within two different hosts. In one host reproduction is asexual in nature, while in the other

it is sexual, as in the plasmodia of malaria, where the cycle of existence in man is asexual and that in the mosquito is sexual.

In sexual reproduction union may occur between two similar organisms, in which case it is said to be *isogamous*, or between two dissimilar organisms, in which case it is said to be *anisogamous*, in which case sexual dimorphism is present, with the formation of *macrogametes* and *microgametes*, the former being the female organisms and the latter the male. The union of microgamete and macrogamete may be permanent, the product of the union being called a *zygote*, or it may be only temporary, when the process is known as *conjugation*. Conjugation, however, is not always reproductive in character, as it may occur between two similar organisms for the purpose of rejuvenation, according to Maupas and others.

In certain of the PROTOZOA the same species may reproduce by simple fission, multiple fission, conjugation, and by budding at different times, and the products of these various methods of reproduction may be very different in morphology. Reproduction by budding, in which small portions of the nuclear substance and cytoplasm are budded off from the surface of the organism, has been described in many species and for a long time was believed to be a method of reproduction in some of the parasitic amœbae of man, as *Endamoeba histolytica*, but it is now believed that, in this instance, the supposed budding was a degenerative process.

The method of reproduction of the PROTOZOA will be more fully discussed in the descriptions of the individual organisms.

**Protozoa as Parasites.**—The various protozoan organisms that live within man or other animals are known as *parasitic protozoa*. The term "parasite" as used in this work follows the definition of a parasite given by Fantham, Stephens, and Theobald (1916), which is as follows:

"By the term parasites is understood living organisms which, for the purpose of procuring food, take up their abode, temporarily or permanently, on or within other living organisms."

Thus, I regard any organism that derives its food from its host and undergoes all or a part of its life-cycle within or upon a host, as a parasite, and all such parasites that are protozoan in nature as parasitic protozoa.

This broad and, from a medical standpoint, practical definition of what constitutes a parasite, is not concurred in by Dobell and O'Connor (1921), who limit the term "parasite" to organisms which live at the expense of their hosts and interpret this to mean that the organism must live upon the tissues of its host. They exclude from the definition organisms which live in man and procure their nourishment from the food eaten by the host or which live upon waste food products and bacteria in the intestine of man. This rigid interpretation of parasitism is unsatisfactory, so far as medical science is concerned, and would lead

to the utmost confusion if applied, especially in the case of the bacteria. One has only to attempt to apply this definition of parasites in the case of vegetable parasites to understand the almost limitless confusion which would be the result of its application. In fact, it is doubtful if any two authorities would agree as to the parasitic nature of any of the bacteria living in man. Harmless commensals are not included by Dobell and O'Connor among true parasites, although they live within man and are dependent upon him for existence. Such an interpretation of the nature of parasites is too limited and, as stated, the definition of the term as given by Fantham, Stephens, and Theobald will be followed in this work in the discussion of the various parasitic protozoa of man.

Parasites are divided into *temporary* parasites or *ectoparasites* or *epizoa* and *permanent* parasites or *endoparasites* or *entozoa*. The former obtain their nourishment from the surface of the body of their host, but do not live permanently upon the host, while the latter obtain their nourishment from their host and live in his fluids or tissues, or within his body, for considerable periods or during their entire life-cycle. Examples of temporary parasites are the flea and bed-bug, while the malaria plasmodia and the parasitic amœbæ are examples of permanent parasites. All the parasitic protozoa of man are permanent or entozoic parasites.

As regards their *relation to man*, the parasitic protozoa may be divided into *pathogenic* and *non-pathogenic* organisms. The pathogenic protozoa include all organisms that produce evident lesions or symptoms of disease, while the latter class of organisms are generally referred to as *commensal parasites* or "harmless commensals." These organisms procure their food from man and live within him, but are harmless to their human host. Examples of the pathogenic protozoa are *Endamæba histolytica*, *Trypanosoma gambiense*, *Leishmania donovani*, the malaria plasmodia, and *Balanitidium coli*, while examples of the non-pathogenic protozoa are *Endamæba coli*, *Trichomonas hominis*, and *Chilomastix mesnili*.

Besides the harmful, or *pathogenic* parasites, and the harmless, or *commensal* parasites, a third class is recognized known as *symbiotic* parasites, in which the association of the host and parasite is mutually beneficial. None of the protozoan parasites of man belong to this class, although Dobell and O'Connor (1921) suggest that it is possible that some of the non-pathogenic protozoa of the intestine of man may be beneficial to their host by consuming waste products and bacteria. This supposition is not supported by any evidence of scientific value and there is no reason to believe that any protozoan parasite of man is actually beneficial to its human host.

There are certain parasitic protozoa that still occupy a questionable position as regards pathogenicity. Of these the most important are the intestinal flagellates, especially *Giardia intestinalis*, *Chilomastix mesnili*,

and *Trichomonas hominis*. Many authorities believe that these flagellates are harmless commensals, while other authorities are equally positive in their belief that they are pathogenic parasites. At present it cannot be said that this question is definitely settled, but the opinion is growing that, when the intestine is in a normal condition, these flagellates are harmless, but that when it is in an inflammatory condition these parasites, when present in large numbers, increase and prolong the inflammatory reaction. This belief appears reasonable when one remembers the very active movements of these flagellates and the enormous number frequently observed in the fæces in diarrhœal conditions.

Some of the most important infections of man, as amœbic dysentery, sleeping sickness, kala-azar, and the malarial fevers, are due to parasitic protozoa, so that a knowledge of the biology, morphology, prophylaxis, and diagnosis of these parasites is of the greatest importance to the physician and public health officer.

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## CHAPTER II

### THE PARASITIC AMŒBÆ OF MAN. HISTORY AND CLASSIFICATION. GENERAL DESCRIPTION. THE GENUS *ENDAMŒBA*. *ENDAMŒBA HISTOLYTICA*.

The parasitic amœbæ belong to the PHYLUM RHIZOPODA (SARCODINA) and order Gymnamœbia, or naked amœbæ. They are typical non-cellular organisms, the body consisting of a single cell, and are very widely distributed geographically. The group contains many species that are parasitic in man and the lower animals but only one species, *Endamœba histolytica*, that is known to be a pathogenic parasite of man.

**Historical.**—The first description of a parasitic amœba of man was published in 1849, by Gros, who discovered and described the common amœba of the human mouth which is now known as *Endamœba gingivalis*. According to Dobell (1919), the first investigators to observe amœbæ in the fæces of man were Lewis (1870) and Cunningham (1871), who found them in the stools of cholera patients in India. The credit for this discovery is generally given to Lambl (1860) but Dobell has shown that Lambl's "amœbæ" were probably degenerating forms of *Trichomonas hominis*.

In 1875, Lösch described an amœba occurring in the fæces of a patient suffering from dysentery, in Russia, and named it *Amœba coli*. This amœba is now known to be identical with *Endamœba histolytica*. Prior to the observations of Lösch, the amœbæ observed in the fæces of man were believed to be harmless commensals in the human intestine but his observations awakened interest in the question of the pathogenicity of these parasites and stimulated the study of intestinal parasites in general, especially as regards their relation to disease.

In 1886, Kartulis began the publication of a series of papers upon his investigations of dysentery as observed in Egypt, studying the amœbæ observed in many hundred cases of the disease, and concluded that the amœbæ were the cause of dysentery and that this form of dysentery was often accompanied by liver abscess and that the amœbæ were found in the pus in such abscesses. In 1887, Hlava, in Prague, published his observations upon the amœbæ he had observed in sixty cases of dysentery, and produced dysentery in dogs and cats by injecting fæces containing the amœbæ into the rectum. Osler (1890) was the first to observe amœbæ in a dysentery patient in America. In 1891, Councilman and Lafleur published their classical monograph upon amœbic dysentery, in which, for the first time, the disease was recognized as a clinical entity, characterized by definite pathological lesions due to the amœbæ. They pro-

posed the name *Amœba dysenteria* for the amœbæ occurring in their cases and it is now recognized that the amœbæ they studied are identical with *Endamœba histolytica*. In their monograph they clearly state that other, and perhaps non-pathogenic, amœbæ may infest the intestine, so that to these authors belongs the credit of being the first to recognize that the amœbæ found in the intestine of man are not all of one species. Quincke and Roos (1893) were the first to prove that more than one species of amœba is parasitic in the intestine of man and described two species, one causing dysentery, and the other a harmless parasite. These authors proved this contention by producing dysentery experimentally in cats with the dysentery amœbæ of man and were unable to produce any symptoms or lesions with the amœba that they regarded as a distinct and harmless species.

In 1894, Kruse and Pasquale, who studied amœbic dysentery in Alexandria, Egypt, confirmed the work of Councilman and Lafleur and Quincke and Roos, and concluded that there existed in the intestine of man two species of amœbæ, one pathogenic and the other a harmless commensal. They produced the disease in cats by the injection into the rectum of material from an abscess of the liver in man containing the amœbæ. Celli and Fiocca (1895), on the other hand, concluded that none of the amœbæ occurring in man were pathogenic, and their conclusion was confirmed by the publications of Casagrandi and Barbagallo (1897). In 1901, Harris produced dysentery in young dogs by rectal injection of material containing amœbæ from dysentery patients and in 1900, Strong and Musgrave recognized two species of amœbæ in man, one pathogenic, the other a harmless commensal. Unfortunately Musgrave, in his later publications, adhered to the belief that all amœbæ might become pathogenic under certain conditions and refused to recognize the existence of pathogenic and non-pathogenic species. This attitude on the part of a recognized authority hindered very greatly the acceptance of Schaudinn's two species, which are now recognized by all protozoologists. In 1902, Jürgens called attention to the existence of a pathogenic and non-pathogenic amœba in the intestine of man and clearly recognized the principal morphological distinctions between the two species.

Although Quincke and Roos, Kruse and Pasquale, and Jürgens had recognized two species of amœbæ parasitic in man, their conclusions were by no means widely accepted, and it was not until the publication of the observations of Schaudinn that most authorities were willing to recognize the existence of pathogenic and non-pathogenic species of amœbæ living in man. In 1903, Schaudinn published a paper giving the results of his work at Rovigno, in which he concluded that there occur in man at least two species of amœbæ, one, a harmless commensal,

occurring in the intestine in both health and disease, the other, the cause of dysentery. Because of his great reputation, Schaudinn's conclusions were generally accepted and were soon confirmed by numerous observers.

Since Schaudinn's work was published great advances have been made in the study of the parasitic amœbæ. The final proof of the existence of the two species repeatedly described by different authors was given by Walker and Sellards (1913), who produced dysentery in man by feeding material containing the cysts of *Endamœba histolytica*, the pathogenic amœba, while they were unable to produce any symptoms or lesions of disease by feeding the cysts of *Endamœba coli*, the harmless amœba, and these authors were the first to call attention to the importance of the "carrier" in the transmission of amœbic infection. New and valid species have been discovered, and we now recognize, in addition to *Endamœba histolytica* and *Endamœba coli*, three other species, i.e., *Endamœba nana*, discovered by Wenyon and O'Connor, in 1917; *Iodamœba williamsi*, discovered by Prowazek, in 1912; and *Dientamœba fragilis*, discovered by Jepps and Dobell, in 1917, and described by them in 1918. At the present time, while the classification and nomenclature of these amœbæ is not agreed upon by all protozoologists, there is no doubt regarding the existence of the five species mentioned and it is only a question of time before some classification will be adopted which will be acceptable to all.

**Classification and Nomenclature.**—The classification of the parasitic amœbæ has always been a most difficult problem owing to our lack of knowledge of the complete life-cycle of many of the so-called species; the very simple morphology of these parasites; the difficulties inherent in the study of such delicate cells with the staining methods at our command; and the conflicting opinions of eminent protozoologists as to the data upon which generic and specific classification should be based. I believe that it may be truthfully stated that none of the classifications so far published can be considered as final, for all must remain tentative in the present incomplete state of our knowledge concerning the organisms in question and the difference in opinion that exists as to what morphological and biological characteristics shall be used for the determination of genera and species. At the present time many authorities regard slight differences in the structure of the nucleus to be sufficient upon which to base even generic differences, an opinion with which I am not in accord, as will be noted later, while others insist that only very evident differences in the life-cycle should be used in the determination of genera and species. It is not my intention here to consider *in extenso* the many classifications of the amœbæ, including the parasitic species, that have been proposed, from time to time, but will only consider those proposed by Dobell (1919), and Stiles and Boeck (1923).

Dobell (1919) places all the parasitic amœbæ of man in four genera, as follows: Genus I, *Entamœba*, Casagrandi and Barbagallo, 1895; Genus II, *Endolimax*, Kuenen and Swellengrebel, 1917; Genus III, *Iodamœba*, Dobell, 1919; Genus IV, *Dientamœba*, Jepps and Dobell, 1918. He recognizes five species of parasitic amœbæ classified as follows:

**Genus I.**—*ENTAMŒBA*, Casagrandi and Barbagallo, 1895, *nec Endamœba*, Leidy, 1879.

Synonyms:

*Poneramœba*, Lühe, 1908.

*Löschia* }  
*Viereckia* } Chatton and Lalung-Bonnaire, 1912.

*Proctamœba*, Alexeieff, 1912.

Type: *E. coli* (Grassi), Casagrandi and Barbagallo.

Species in man: *E. coli* (Grassi), Casagrandi and Barbagallo.

*E. histolytica*, Schaudinn (*emend* Walker).

*E. gingivalis* (Gros), Brumpt.

**Genus II.**—*ENDOLIMAX*, Kuenen and Swellengrebel, 1917.

Only species, hence type: *E. nana* (Wenyon and O'Connor), Brug.

**Genus III.**—*IODAMŒBA*, Dobell, 1919.

Only species, hence type: *I. bütschlii* (Prowazek), Dobell.

**Genus IV.**—*DIENTAMŒBA*, Jepps and Dobell, 1918.

Only species, hence type: *D. fragilis*, Jepps and Dobell.

In his classification Dobell accepts the generic name *Entamœba*, established by Casagrandi and Barbagallo (1895), who were apparently in ignorance of Leidy's genus *Endamœba*, established in 1879, to include the parasitic amœba of the cockroach (*Blatta orientalis*). It is more than probable that had these authors known of Leidy's designation of *Endamœba* as a generic name for a parasitic amœba they would have adopted it, instead of *Entamœba*, to include the parasitic amœba of man, *E. coli*.

The latest classification of the parasitic amœbæ published is that of Stiles and Boeck (1923), who recognize the generic name *Endamœba* instead of *Entamœba*, and two subgenera within this genus, *i.e.*, *Endamœba* and *Poneramœba*, the subgeneric names not to be used in combination at the present time. The classification of Stiles and Boeck follows:

**Genus I.**—*ENDAMŒBA*, Leidy, 1879.

1st Subgenus, *Endamœba*, Leidy, 1879.

Type. *E. blattæ*, Leidy, 1879.

2nd Subgenus, *Poneramœba*, Lühe, 1909 (mt., tod. *E. histolytica*), containing two possible (but as yet not well established) groups:

2': *Poneramœba*, Lühe, 1909 (mt. *E. histolytica*), Syn. *Viereckia*.

2'': *Löschia*, Chatton and Lalung-Bonnaire, 1912 (tod. *E. coli*), Syn. *Entamœba*. *Proctamœba*.

3rd Subgenus. (Not yet named.) May perhaps be necessary to include *E. ranarum* as type.

Species in Man: *E. histolytica* (Schaudinn, 1903), Hickson, 1909.

*E. coli* (Lösch, 1875), Hickson, 1909.

*E. gingivalis* (Gros, 1849), Smith, Middleton, and Barrett, 1914.



**Genus II.—ENDOLIMAX**, Kuenen and Swellengrebel, 1917.

Species in Man: *E. nana* (Wenyon and O'Connor, 1917), Kofoid and Swezy, 1917. Type.

*E. williamsi* (Prowazek, 1911), Stiles and Boeck, 1923.

**Genus III.—DIENTAMŒBA**, Jepps and Dobell, 1918.

Species, *Dientamæba fragilis*, Jepps and Dobell, 1918. Type.

**Genus IV.—COUNCILMANIA**, Kofoid and Swezy, 1921. Genus inquirendum.

Species, *Councilmania lafleuri*, Kofoid and Swezy, 1921.

The classification of Stiles and Boeck is an improvement over that of any hitherto published in that it enables all authors to agree upon the one generic name *Endamæba* and still recognize differences in the parasitic amœbæ that are greater than specific differences but less than generic.

From my own observations I do not believe that the genus *Endolimax* should be recognized, but that it should be regarded as a subgenus of the genus *Endamæba*, for the reason that *Endolimax nana* does not, in my opinion, differ enough either in morphology or life-history from the amœbæ classed in the genus *Endamæba* to entitle it to be the type of a new genus. On the other hand, I believe that Dobell's genus *Iodamæba* is valid, and in this work I shall regard the genus *Endolimax* as a subgenus of *Endamæba* and *Iodamæba* to be a valid genus.

The classification of the parasitic amœbæ that I would propose, with the understanding that the subgeneric names will not be used in combination at present, is the following:

**Genus I.—ENDAMŒBA**, Leidy, 1879.

1st Subgenus, *Endamæba*, Leidy, 1879. Type, *Endamæba blattæ*.

2nd Subgenus, *Poneramæba*, Lühe, 1908. Type, *Endamæba histolytica*.

3rd Subgenus, *Endolimax*, Kuenen and Swellengrebel, 1917. Type, *Endamæba nana*.

Species in Man: *Endamæba histolytica* (Schaudinn, 1903), Hickson, 1909.

*Endamæba coli* (Grassi, 1879), Hickson, 1909.

*Endamæba gingivalis* (Gros, 1849), Smith, Middleton, and Barrett, 1914.

*Endamæba nana* (Wenyon and O'Connor, 1917), Craig, 1921.

**Genus II.—IODAMŒBA**, Dobell, 1910.

Species, *Iodamæba williamsi* (Prowazek, 1911), Taliaferro and Becker, 1922. Type.

**Genus III.—DIENTAMŒBA**, Jepps and Dobell, 1918.

Species, *Dientamæba fragilis*, Jepps and Dobell, 1918. Type.

**Genus IV.—COUNCILMANIA**, Kofoid and Swezy, 1921. Genus inquirendum.

Species, *Councilmania lafleuri*, Kofoid and Swezy, 1921. Type.

It is believed that the classification proposed above is adequate so

far as our present knowledge of the parasitic amœbæ is concerned and that, from a medical standpoint, it is more practical, in that all of the most common parasitic amœbæ of man are placed in the genus *Endamæba*, while recognition is accorded the differences between the various species which are superspecific but subgeneric.

**General Description.**—The parasitic amœbæ of man, like all amœbæ, consist of a mass of protoplasm which contains a nucleus or nuclei, and food vacuoles. Contractile vacuoles are not present in any species of parasitic amœba of man, or the lower animals, although Leidy stated, in his description of *Endamæba blattæ*, the parasitic amœba of the cockroach, that contractile vacuoles were sometimes present. These amœbæ vary considerably in size, but they are all small organisms compared with some of the free-living species. The size varies, at different stages of development, from five microns to as much as seventy or eighty microns in diameter, when resting, but as their shape is constantly changing, due to the prolongation of pseudopodia, the size varies accordingly.

The cytoplasm of all parasitic amœbæ is divided into a thin outer portion, the ectoplasm, and an inner portion, the endoplasm, which contains the nucleus and food vacuoles. In some species the ectoplasm is well defined, as in *Endamæba histolytica*, even when the organism is very sluggishly motile, while in others the ectoplasm is poorly defined from the endoplasm, as in *Endamæba coli*. The nucleus is visible in some species and invisible in others, unless stained preparations be examined. The nucleus varies considerably in structure in different genera and species, and this variation is used in the differentiation of both genera and species to an unwarranted extent by some authorities. Besides the nucleus the endoplasm contains food vacuoles in which ingested material used as food may be observed, as bacteria, crystals, etc. Besides the food vacuoles the endoplasm may contain chromidial bodies, crystals, other protozoan organisms, leucocytes, or red blood corpuscles, as in the case of *Endamæba histolytica*.

Motility is accomplished by means of pseudopodia formed by the ectoplasm, and the morphology of these pseudopodia is of some assistance in the differentiation of species. For instance, the finger-shaped, clear, glass-like pseudopodia of *Endamæba histolytica* are quite different from the blunt, veil-like, and smaller pseudopodia of *Endamæba coli*, and are of much diagnostic value in the differentiation of the two species. The pseudopodia also serve to procure food for the amœbæ by flowing around and engulfing the food particles.

Reproduction in the parasitic amœbæ may be by simple fission or by multiple fission within a cyst. Reproduction by budding was formerly

believed to be characteristic of *Endamæba histolytica*, but it is now recognized that the supposed budding forms were really degenerating amœba. In *Councilmania lafleuri*, described by Kofoid and Swezy, budding is stated to be a method of reproduction, but their observations await confirmation.

The cysts are developed from the vegetative forms, or trophozoites, and differ markedly from them in morphology. In most species multiple division of the nucleus occurs within the cyst, and the number of nuclei in the cysts is a valuable differential character as between species. Thus, the cysts of *Endamæba histolytica* contain only four nuclei when fully developed, while those of *Endamæba coli* contain eight nuclei when fully developed. The cysts of the parasitic amœbæ, as long as they are kept moist and are not exposed to great extremes of heat and cold, will live for a long time outside of the body of the host, and are the infective agents, as infection does not occur from swallowing the vegetative forms, or trophozoites, which cannot withstand the action of the gastric secretions. When the cysts are swallowed by a suitable host they pass through the stomach unharmed, by reason of the resistant cyst wall, but when they reach the intestine the cyst wall is dissolved or broken in some manner and the young amœbæ are liberated. So far as is known reproduction in the parasitic amœbæ is always asexual in character, and there is no alternation of generations and hosts. Conjugation has been described by many authorities. It is still uncertain whether it occurs in any of the parasitic amœbæ, but there is good reason to believe that it does occur under certain conditions, resulting in a rejuvenescence of the vital processes, as suggested by Maupas and others.

The parasitic amœbæ may be studied in the living condition, in stained preparations, and in stained sections of tissue which may contain them, as in infections with *Endamæba histolytica*. One who is trained in the study of these amœbæ is able to differentiate some of the species by a study of the living specimens, but the use of some method of staining is often necessary in order to differentiate one species from another, and is absolutely necessary for the study of the character of the nucleus in the trophozoites and in the cysts. This subject will be found fully discussed in the section dealing with the diagnosis of the parasitic amœba of man, and the methods which have been found most useful in fixing and staining the amœbæ are given in detail in the Appendix.

### Genus I. ENDAMŒBA Leidy, 1879.

Synonyms: *Entamæba*, Casagrandi and Barbagallo, 1895. *Poneramæba*, Lühe, 1908. *Löschia*, Chatton and Lalung-Bonnaire, 1912. *Vireckia*, Chatton and Lalung-Bonnaire, 1912. *Proctamæba*, Alexeieff, 1912.

The genus *Endamæba* was established by Leidy, in 1879, to include

the parasitic amœba of the cockroach (*Blatta orientalis*). Prior to Leidy's researches all amœbæ, both free-living and parasitic, were included in the genus *Amœba*, but his studies of *Amœba blattæ* Bütschli, the parasitic amœba of the cockroach, convinced him that it should be placed in a new genus distinct from the free-living amœbæ with which it had been classified, because of marked differences in morphology, life-cycle, and habitat, and he proposed the name *Endamœba* for this new genus. Thus *Amœba blattæ* became *Endamœba blattæ* and the type species of the genus.

Casagrandi and Barbagallo (1895), in ignorance of Leidy's genus *Endamœba*, proposed the name *Entamœba* for a new genus in which *Entamœba coli* was selected as the type species. This error was repeated again in 1903, by Schaudinn, who accepted Casagrandi and Barbagallo's name, and included in this genus both *coli* and *histolytica*.

The question as to whether *Endamœba blattæ* is cogenetic with *Endamœba histolytica* and *Endamœba coli* is still in dispute, and until the question is settled it is the part of wisdom to place all of these amœbæ in the genus *Endamœba* and to drop the name *Entamœba* entirely from use as a generic name. As shown in the list of synonyms, various authorities have suggested other generic names for this genus. I am in agreement with Stiles that the name *Poneramœba* should be the name of a subgenus, as I am convinced that the differences between *Endamœba blattæ* and *Endamœba histolytica* or *Endamœba coli* are not great enough to warrant placing the two latter species in a different genus although greater than the usual differences between species of amœbæ, and, therefore, sufficient to place them in a subgenus of *Endamœba*. Likewise, I believe that the genus *Endolimax* should be considered a subgenus of *Endamœba* and that the proper name of *Endolimax nana* is *Endamœba nana*, for the same reasons. Of course this is purely a matter of opinion, but it is based upon good morphological and biological evidence and greatly simplifies our conception of the classification of these parasites.

The genus *Endamœba*, as shown in the classification upon page 15, contains three subgenera, *i.e.*, *Endamœba*, *Poneramœba*, and *Endolimax*. Owing to our limited knowledge at the present time the subgeneric names should not be used in combination and all of the amœbæ belonging to these subgenera should be called by the generic name *Endamœba*. There are four species of parasitic amœbæ in man belonging to this genus, *i.e.*, *Endamœba histolytica*, *Endamœba coli*, *Endamœba nana*, and *Endamœba gingivalis*.

The reader is referred to the very excellent monographs of Dobell (1919) and of Boeck and Stiles (1923) for a thorough discussion



of the complicated nomenclature of the genera and species of the parasitic amœbæ of man.

Species I. *ENDAMŒBA HISTOLYTICA* (Schaudinn, 1903),  
Hickson, 1909.

The synonyms of *Endamœba histolytica* are very numerous, as many supposedly new species that have been described, from time to time, have been found to be identical with this species. The most important are the following:

Synonyms: "Amœba coli," Lösch, 1875. "Amœba dysenteriae," Councilman and Lafleur, 1891. *Entamœba histolytica*, Schaudinn, 1903. *Entamœba dysenteriae* (Councilman and Lafleur), Craig, 1905. *Entamœba africana*, Hartmann, 1907. *Entamœba tetragena* (Viereck), Hartmann, 1908. *Poneramœba histolytica*, Lühe, 1908. *Entamœba minuta*, Elmassian, 1909. *Entamœba nipponica*, Koidzumi, 1909. *Entamœba hartmanni*, Prowazek, 1912. *Löschia (Viereckia) tetragena*, Chatton and Lalung-Bonnaire, 1912. *Entamœba brasiliensis*, Aragão, 1912. *Löschia histolytica* (Schaudinn), Mathis, 1913. *Entamœba venaticum*, Darling, 1915. *Endamœba coli* (Lösch), Aragão, 1917. *Endamœba dysenteriae* (Councilman and Lafleur), Pestana, 1917. *Entamœba tenuis*, Kuenen and Swellengrebel, 1917. *Entamœba histolytica* (Schaudinn), Craig, 1917. *Endamœba dysenteriae*, Kofoid, 1920.

**History and Nomenclature.**—The amœba now known as *Endamœba histolytica* was first described by Lösch, in 1875, who found the parasite in the stools of a Russian, in St. Petersburg, who was suffering at the time from a serious attack of dysentery. From the description of Lösch it is perfectly evident that the amœba he studied and described was *Endamœba histolytica* and, furthermore, he was able to produce an attack of dysentery in a dog by injecting material containing the amœbæ *per rectum* and the same amœbæ were found in the stools of the dog after the development of dysentery. At autopsy, the dog showed ulcerations in the intestine, and similar ulcerations were found at autopsy in the intestine of the Russian. Lösch did not regard the amœbæ as the cause of the dysentery but suggested that they might, by their presence, prevent the healing of the ulcerations. He named the parasite "Amœba coli," a name by which it was known in medical works until Schaudinn's contribution appeared. Whether Lösch was dealing with a mixed infection of *Endamœba histolytica* and *Endamœba coli* is, so far as evidence goes, a matter of opinion, but there is no definite evidence in his description that warrants such an opinion.

The occurrence of amœbæ in the stools and lesions of patients suffering from dysentery was confirmed by Koch (1883), Kartulis (1885), Osler (1890), Councilman and Lafleur (1891), Kovacs (1892), Quincke and Roos (1893), Kruse and Pasquale (1894), and Harris (1901). During the period covered by the reports of these investigators the following facts were established regarding *Endamœba histolytica* and its relation to disease:

1. That amœbæ corresponding to those described by Lösch occurred in the stools and intestinal lesions of patients suffering from a form of dysentery that Councilman and Lafleur (1891) called amœbic dysentery.

2. That similar amœbæ also occurred in the contents of the liver abscesses that often accompanied amœbic dysentery. Kartulis (1887), Osler (1890), Councilman and Lafleur (1891).

3. That amœbæ occurred in the stools of healthy individuals and those suffering from diseases other than dysentery.

4. That the injection of fæces containing the amœbæ *per rectum* into cats and puppies from cases of dysentery resulted in the production of dysentery in the experimental animals. Lösch (1875), Hlava (1887), Kovacs (1892), Kruse and Pasquale (1894), Quincke and Roos (1893), and Harris (1901).

5. That the rectal injection of amœbæ from the pus of amœbic abscesses of the liver into cats produced dysentery in the experimental animals. Kruse and Pasquale (1894).

6. That the feeding or rectal injection of puppies with material containing the amœbæ or cysts present in the fæces in dysentery cases was sometimes followed by the appearance of abscess of the liver. Harris (1901). Later abscess of the liver was produced in this manner in cats by Craig (1905), Huber (1909), Wenyon (1912), Bætjer and Sellards (1914), and Dale and Dobell (1917).

While these observations were sufficient to prove that the amœba described by Lösch was the cause of amœbic dysentery and of the liver abscesses frequently associated with that disease, the fact that amœbæ also occurred in the stools of healthy individuals and of persons suffering from other diseases than dysentery prevented many authorities from accepting amœbæ as the cause of the disease. In order to explain the facts some authorities, as Councilman and Lafleur (1891) and Quincke and Roos (1893), suggested that there might be pathogenic and non-pathogenic amœbæ parasitic in the human intestine, and to the latter authors we owe the first clear description of the morphological differences between the pathogenic amœba now known as *Endamæba histolytica* and the non-pathogenic amœba now known as *Endamæba coli*. Not only did Quincke and Roos recognize the differences between the vegetative forms of the two species, but they also were the first to describe the cysts of *Endamæba histolytica* and to prove that cats could be given dysentery by feeding material containing the cysts, or by rectal injection of the motile forms. The importance of the researches and results of these authors was overlooked, and it was not until the publication of the work of Schaudinn (1903) that the distinction between *Endamæba histolytica* and *Endamæba coli* attracted general attention.

In 1903, Schaudinn published the results of his researches upon the

amœbæ occurring in the intestine of man, in which, in evident ignorance of the genus *Endamæba*, established by Leidy, in 1879, he accepted Casagrandi and Barbagallo's genus *Entamæba* for these amœbæ and recognized two species. One of these, the cause of dysentery, he named *Entamæba histolytica*, while the other, a harmless commensal in the intestine of man, he named *Entamæba coli*. He gave good descriptions of the morphology of the trophozoites, or vegetative stage of these amœbæ, but entirely overlooked the cysts of *Endamæba histolytica* and erroneously described the method of reproduction in this species as being by gemmation or budding. Schaudinn's great authority as a zoologist led to the general acceptance of his classification and descriptions, and it was not until the paper of Walker (1911) appeared that the method of reproduction described for *Endamæba histolytica* by Schaudinn was shown to be erroneous, and that this species formed cysts containing four nuclei when fully developed.

Quincke and Roos (1893) were the first to describe the cysts of *Endamæba histolytica*, but, as stated, little attention was paid to their discovery. The cysts were rediscovered by Huber (1903), who also produced dysentery in a cat by feeding it material containing the cysts, but his work was also unrecognized and the statements of Schaudinn as to the method of reproduction of this species undoubtedly influenced most workers to ignore the observations of both Quincke and Roos and Huber. In 1907, Viereck redescribed the cysts of *Endamæba histolytica* and considered that they belonged to a new species, and Hartmann (1907) also redescribed them and also believed that they were the cysts of a new species which was named *Entamæba tetragena*. This supposed new species was confirmed by numerous investigators, but Walker (1911) demonstrated conclusively that *Entamæba tetragena* is identical with *Endamæba histolytica*, that the latter species does not reproduce by budding, and that the cysts described as those of *Entamæba tetragena* were, in reality, those of *Endamæba histolytica*. Walker's conclusions have been confirmed by all students of these parasites and are now generally accepted.

In 1913, Walker and Sellards proved by actual experiments upon man that *Endamæba histolytica* is the cause of amœbic dysentery, and that *Endamæba coli* is a harmless commensal of the human intestine. They also called attention to the importance of "carriers" in the transmission of amœbic infections.

**Nomenclature.**—The nomenclature of *Endamæba histolytica* will not be discussed *in extenso* here. Hundreds of pages have been written upon the subject, and there is still no general agreement regarding it. To those who are interested the most excellent discussions contained in the monographs of Dobell (1919) and of Boeck and Stiles (1923) are recommended, but it may be stated that it is the consensus of opinion

of recent writers that the specific name of the amœba causing dysentery should be *histolytica*, as proposed by Schaudinn, and that of the most common harmless amœba of man should be *coli*, as proposed by Casagrandi and Barbagallo. The generic status of these amœbæ has already been discussed, and it is my belief that both parasites should be placed in the genus *Endamœba* and that the name *Entamœba* should be dropped as a generic name.

As will be noted in the list of synonyms, many authorities have described as new species organisms which were really identical with *Endamœba histolytica*. Thus *Entamœba tetragena*, *Entamœba nipponica*, *Entamœba minuta*, *Entamœba hartmanni*, *Entamœba venaticum*, *Entamœba brasiliensis*, and *Entamœba tenuis* are all identical with *Endamœba histolytica*, and these names all become synonyms of the latter species.

**Morphology.**—*Endamœba histolytica* has three distinct stages in its life-cycle which are well known, and in each of which it varies in morphology. These stages are the vegetative stage, the pre-cystic stage, and the cystic stage. The morphology of the parasite in each of these stages will be described in both living and stained preparations.

1. **Vegetative Stage.**—*a. Living Preparations.* In the living condition the size of *Endamœba histolytica* varies greatly. Lösch stated that the rounded forms varied in diameter from 20 to 30 microns; Dobell (1919) gives the diameter as from 18 to 40 microns; and Boeck and Stiles (1923) state that the forms found in the stools in acute dysentery vary from 20 to 30 microns in diameter. My own observations, which cover the study of this species in hundreds of cases of amœbic dysentery, have shown that the diameter of the round immotile vegetative forms varies from 15 to as much as 80 microns, although the latter measurement is very rarely reached. However, I have observed these very large amœbæ in the bloody stools of acute cases of dysentery several times, and in every instance they were filled with red blood corpuscles. In my experience the vast majority of amœbæ of this species vary in diameter from 20 to 40 microns, the average organism measuring about 25 microns. I have repeatedly observed cases of amœbic dysentery in which the vast majority of the amœbæ present in the fæces were much smaller than 25 microns, but in which the morphology otherwise was identical with *Endamœba histolytica*, and in such instances it was noted that a small race of cysts was generally present, so that it is my belief that the races of this species that produce the small cysts are also smaller in their motile, vegetative stage than those that produce the large cysts.

When motionless *Endamœba histolytica* is generally round in shape,



but may be oval. When in motion the shape varies greatly, due to the pseudopodia.

The *cytoplasm* of this species is divided into two portions, the *ectoplasm* and the *endoplasm*. The *ectoplasm* is clearly differentiated only when the parasite is moving, as a rule, although I have seen motionless amœbæ of this species that showed a distinct ectoplasm. When moving, the ectoplasm forms the pseudopodia and is very sharply demarcated from the endoplasm when motility is not too pronounced. In such amœbæ the *ectoplasm* appears as a clear, glass-like, rather firm substance very distinctly differentiated from the endoplasm. Under high magnifications it appears to be composed of finely granular material and never contains bacteria or other substances in the motionless organisms.

The *endoplasm*, which comprises more than two-thirds of the body of the parasite, resembles ground glass in appearance, being more coarsely granular than the ectoplasm, and of a grayish tint. The endoplasm contains the nucleus,

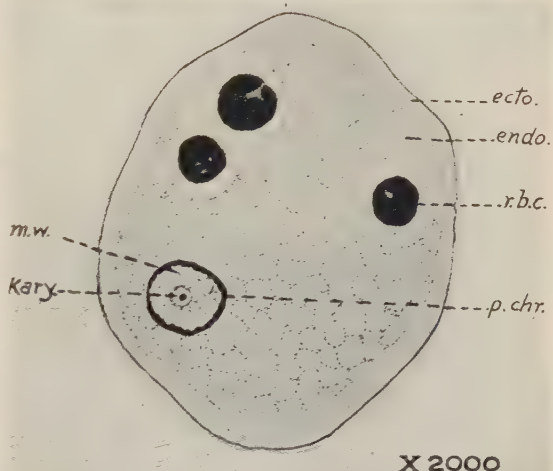


FIG. 2.—*Endamæba histolytica*. X 2,000. (After Dobell and Boeck and Stiles.) Large, motile vegetative form, showing ectoplasm (ecto.), endoplasm (endo.), nucleus with karyosome (kary.), meshwork, or linin net-work (m.w.), peripheral chromatin (p.chr.), and three red blood corpuscles that have been ingested by the organism (r.b.c.).

and vacuoles may be present, but not in amœbæ that have been freshly voided, and it should be remembered that in order to study the normal morphology of this parasite one must examine the stools immediately after passage, as degenerative changes occur very rapidly and the amœbæ in stools that have been voided for even a half-hour will present appearances that are not normal, especially as regards the structure of the nucleus and the occurrence of vacuoles. However, I have observed one or two vacuoles in many amœbæ of this species as soon as they were voided, so that I believe that one or more food vacuoles are frequently present in these organisms. The endoplasm never contains bacteria or crystals except in degenerating organisms, when the endoplasm may be filled with bacteria. If the stools contain blood the endoplasm frequently contains red blood corpuscles which have been ingested by the parasite and sometimes in large numbers, the entire amœba being crowded with these cells. I have counted as many as 36 red blood cor-

puscles within a single amœba of this species, and it is very common to find as many as six or eight within the endoplasm. This property of phagocytizing the red blood corpuscles of its host is a most valuable differential point between this species and the other parasitic amœbæ of the human intestine, a point which I called attention to as long ago as 1905, but which more recent writers have spoken of as though it were unknown before their contributions. The endoplasm may also contain leucocytes or other cells. In many of the amœbæ containing red blood corpuscles the endoplasm has a greenish tint evidently due to the dissolved hæmoglobin from the red cells. I have seen the same greenish tint of the endoplasm in amœbæ in which no red blood corpuscles could be seen, but in cases in which the fæces contained much blood.

The *nucleus* is generally invisible in the living amœbæ, and only becomes visible as the organism degenerates. In the motile amœbæ the nucleus is constantly changing its position, and it is exceedingly difficult to study it at this time owing to its delicate structure and disappearance from view during the movements of the parasite. In resting amœbæ it may sometimes be visible as a very delicate refractile circle of granules situated toward the boundary of the organism, and in degenerate amœbæ it is sometimes visible as a round, refractile mass somewhere within the endoplasm, and in such amœbæ numerous vacuoles may be present and the endoplasm may contain large numbers of bacteria.

*Motility* is very marked in specimens of *Endamæba histolytica* examined in freshly passed fæces. This property is rendered possible by the pseudopodia formed of the ectoplasm and which, in this species, are very clearly differentiated from the endoplasm. The pseudopodia are generally long and finger-shaped, but may be shorter and more blunt, and are generally extruded quite rapidly, the endoplasm immediately flowing into them. In the most active specimens it is impossible to see the ectoplasmic nature of the pseudopodia as the endoplasm flows into them so rapidly that the distinction is lost.

Three forms of motility may be distinguished: active progressive motion, the extrusion of pseudopodia without progression, and movements of the cytoplasm as a whole.

Progressive motion, in freshly voided amœbæ, is always rapid, but as the fæces cool, progressive motion becomes slower and slower and is finally lost. The amœba advances by rapidly extruding the pseudopodium, into which the endoplasm flows immediately, and in these amœbæ the pseudopodium is generally long and finger-like in shape. In the rapidly moving amœba the motion is markedly progressive, the organism flowing along over the microscopic slide in a definite direction, but as motility begins to decrease, the definite direction is lost and the amœba

moves in an irregular course, or first in one direction and then in another.

The second form of motility is frequently observed in the amoebae which have been exposed in the faeces to room temperature for some time, and consists of the extrusion of the pseudopodia unaccompanied by progressive motion. In such amoebae the endoplasm does not flow into the pseudopodia, as a rule, and the pseudopodia can be seen to be composed of the clear, glass-like, hyaline ectoplasm, and are sharply differentiated from the endoplasm.

The third form of motility is only rarely observed and its nature is unknown. It apparently is due to currents within the endoplasm which cause ingested bodies to move about within it in a circular manner, while the border of the parasite shows an undulatory motion. This form of motility may have something to do with encystment, but it is so rare that it is more probable that it is a degenerative phenomenon.

*b. Stained Preparations.* The morphology of *Endamoeba histolytica* is best shown in preparations wet-fixed and stained with one of the haematoxylin methods. The methods employed for fixing and staining are

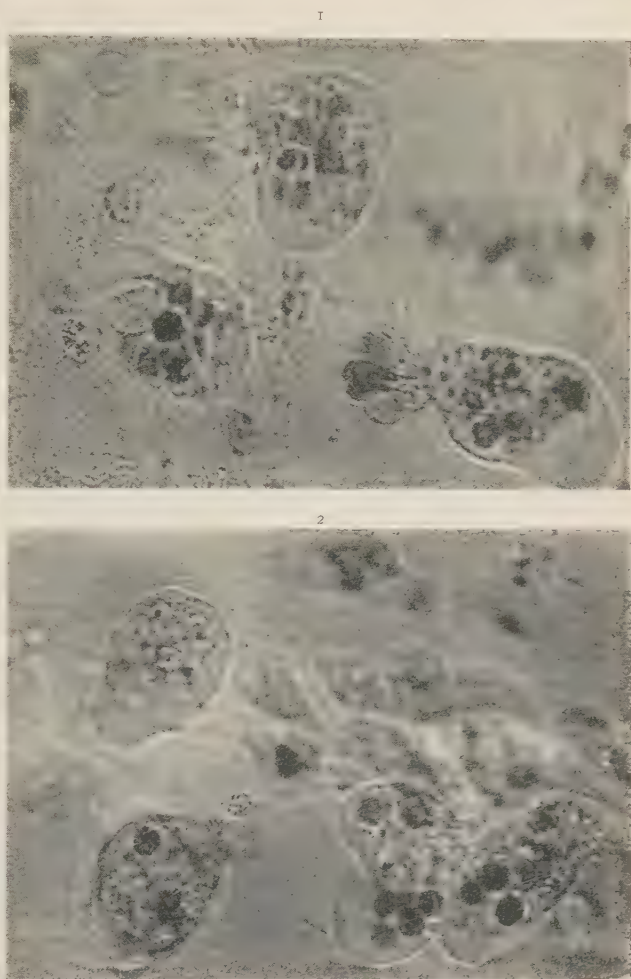


FIG. 3.—*Endamoeba histolytica*.  $\times 750$ . Photomicrographs. Army Medical School Collection.) 1. Motile trophozoites of *Endamoeba histolytica* photographed while moving. The organism in lower right-hand corner shows a pseudopodium which was actively moving while the photograph was taken, thus giving it a divided appearance. Red blood corpuscles are present in all three of the amoebae. 2. Four motile trophozoites of *Endamoeba histolytica*. The three amoebae in line at lower portion of photomicrograph all contained red blood corpuscles. The nucleus is not visible in any of these amoebae.



discussed in the section treating of the diagnosis of the parasitic amœbæ of man and in the Appendix. In order to secure amœbæ presenting typical morphology it is necessary to stain the parasites immediately after the fæces are voided, as otherwise the nuclear structures are greatly altered, and, as stated by Dobell, much of the confusion existing regarding the morphology of the parasitic amœbæ has been due to the study of stained specimens of degenerating organisms. If possible, amœbæ should be obtained from any ulcerations present in the rectum by means of a rectal swab, as such organisms show the most typical morphology.

In material so obtained, or in material obtained at autopsy from the ulcerations, *Endamœba histolytica* presents a most characteristic appearance, especially as regards the structure of the nucleus. The cytoplasm stains a grayish or brownish-gray color, while the structures of the nucleus stain black. The nucleus presents a very delicate nuclear membrane, not over a line in thickness, covered upon its inner surface by a single layer of very minute chromatin granules, either in close apposition or separated by very small intervals. These granules stain intensely black, and are usually of uniform size. At the centre of the nucleus is a very small black dot, the karyosome, measuring about 0.5 micron in diameter, and without a centriole. The karyosome is surrounded by a round, unstained halo which resembles a capsule and which, in degenerating amœbæ, is often stained a grayish-brown color, the so-called *tetragena* type of karyosome.

Between the karyosome and the nuclear membrane, in well-stained preparations, there are delicate strands or threads forming the linin network, which are generally poorly defined. In normal individuals of this species there are never any chromatin granules visible between the karyosome and the nuclear membrane, which distinguishes this species from other intestinal amœbæ. The entire nucleus measures from four to five microns in diameter in stained specimens.

The cytoplasm is free from ingested bacteria unless degeneration is occurring, but red blood corpuscles, leucocytes, and other cells are sometimes observed. The red blood corpuscles do not stain, appearing as greenish-yellow bodies in the cytoplasm.

After the fæces have been voided some time the amœbæ lose their typical nuclear structure due to degenerative changes. In such amœbæ the nuclear membrane appears heavier and the chromatin lining the membrane is in larger, more irregular masses, and arranged irregularly upon the membrane. The karyosome is larger and the unstained halo is absent or appears to be slightly stained, while there is considerable chromatin, in the form of black granules, in the space between karyosome and the nuclear membrane. This is the type of nucleus which was described by so many writers as the *tetragena* type but which is now known to be



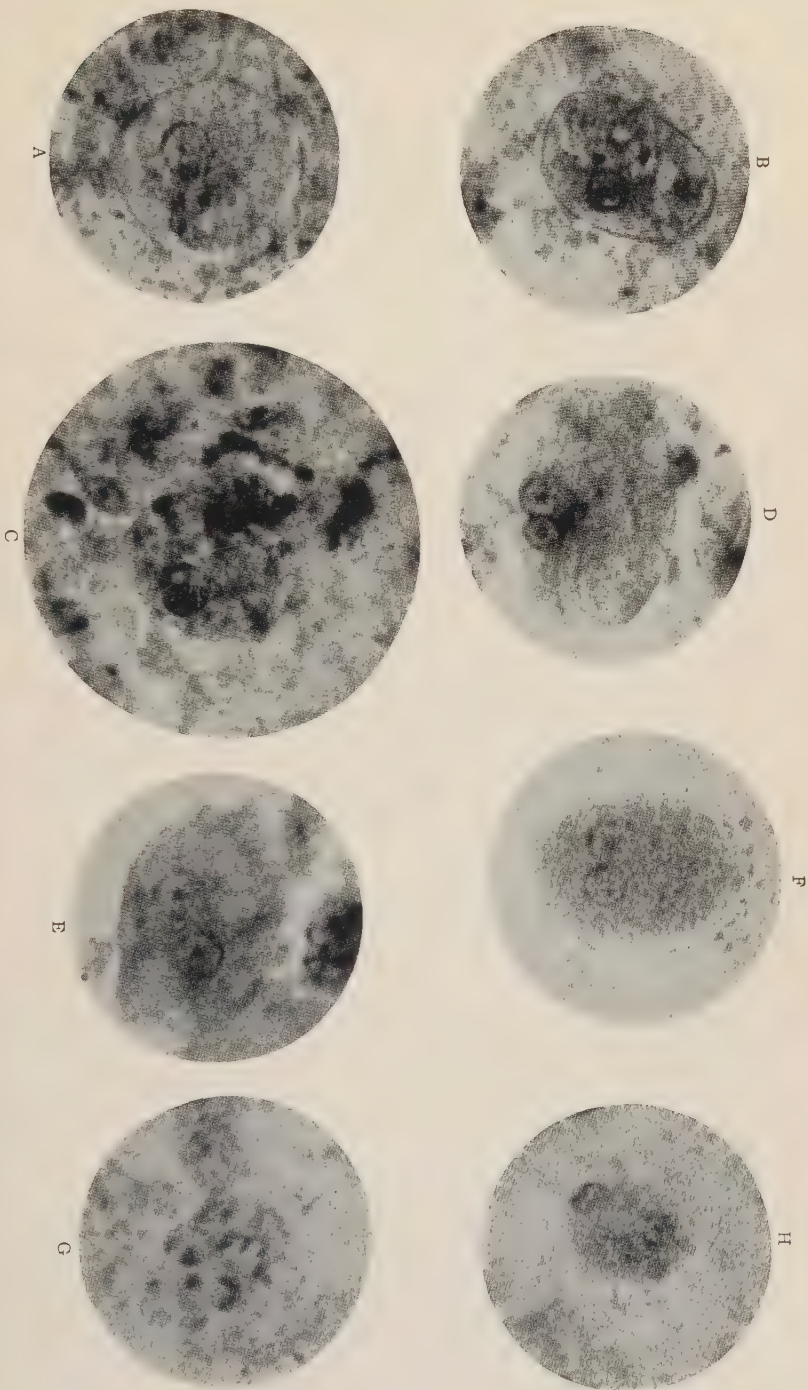


FIG. 4.—*Endamoeba histolytica*. X 1,300. (Photomicrographs. Army Medical School Collection.) Stained with iron-haematoxylin. A. Vegetative form (trophozoite) of *E. histolytica*, showing typical *histolytica* type of nucleus. X 1,300. B. Vegetative form of *E. histolytica*, showing a modified *leishmania* type of nucleus. X 1,300. C. Vegetative form of *E. histolytica*, showing *leishmania* type of nucleus. The karyosome is too deeply stained to show the centriole. X 1,300. D. Vegetative form of *E. histolytica* undergoing simple division. The two new nuclei have just separated and are of a modified *leishmania* type. X 1,300. E. Vegetative form of *E. histolytica* undergoing simple division. This organism is of great interest as it shows one nucleus having a typical *histolytica* structure and one having just as typical a *leishmania* structure. X 1,300. F. Vegetative form of *E. histolytica*. The chromatin is scattered throughout the cytoplasm and some of it is being budded off from the periphery of the parasite. X 1,300. G. Pre-cystic vegetative form of *E. histolytica*. Note reduced size and intermediate type of nucleus, the karyosome being larger than in the large vegetative *histolytica* and smaller than in the *leishmania* type of nucleus. X 1,200.

entirely due to degeneration of the nucleus. This term should no longer be used in referring to the nucleus of *Endamæba histolytica* but, unless one examines absolutely fresh material, the nuclei of the amœbæ will present only the morphology just described, as degeneration occurs very rapidly in the nucleus of *Endamæba histolytica* after removal from the body.

The vegetative, or trophozoite, stage of *Endamæba histolytica* is only found in the stools during the acute symptoms of dysentery, disappearing as the stools return to normal consistence. They are most numerous in the bloody, liquid stools characteristic of the acute stage of the disease, but a few may occur even when the stools are of a semi-fluid nature, although at this time the pre-cystic amœbæ are most numerous. This stage is never found in semi-formed or formed stools, but a saline cathartic will often result in the appearance of motile trophozoites even when the symptoms of dysentery have disappeared.

2. **The Pre-cystic Stage.**—*a. Unstained Preparations.* Prior to encystment the motile forms, or trophozoites, of *Endamæba histolytica* become reduced in size and ingested material, as red blood corpuscles, leucocytes, or food material, is eliminated. The reduction in size is probably accomplished by a series of divisions, and several races have been studied producing cysts of different sizes, so that the size of the pre-cystic forms varies widely.

In the living condition the pre-cystic amœbæ of this species appear as colorless, hyaline, round or slightly oval bodies, when motionless, measuring from 6 to 20 microns in diameter. Motility may be present, but it is sluggish in character and either non-progressive or very slowly progressive.

Usually progressive motion is absent, the organisms slowly protruding pseudopodia, which are withdrawn before the endoplasm flows into them. In contrast with the long, finger-like pseudopodia of the trophozoites, the pseudopodia of the pre-cystic forms are much shorter and more blunt and the distinction between the ectoplasm, of which they are composed, and the endoplasm, is much less marked than in the trophozoites and sometimes almost absent.

The nucleus is often visible in the pre-cystic forms as a ring of small refractile granules lying eccentrically in the cytoplasm or as a refractile mass near the centre of the organism.

The cytoplasm is free from ingested material and appears less refractive than in the trophozoites. Large refractile bodies are sometimes present in the cytoplasm which resemble the chromidial bodies observed in the cysts and which are undoubtedly identical with them. They are spindle-, bar-, or rod-like bodies, with rounded ends.

The pre-cystic forms eventually become cysts within the intestine,

but if they are voided in the pre-cystic condition encystment does not occur and they degenerate and perish within a few days.

*b. Stained Preparations.* In preparations stained, after wet-fixation, with the hæmatoxylin stains, the cytoplasm stains a grayish-brown, and the nucleus is well differentiated, some of the structures of which it is composed staining black. The nuclear membrane appears slightly thicker than in the trophozoites and the karyosome proportionately larger. Small granules of chromatin may be present between the karyosome and the nuclear membrane, lying upon the linin net-work. The karyosome is usually central in position, although it may be slightly eccentric in some of the amœbæ.

At this stage in its development, *Endamœba histolytica* resembles the same stage in the development of *Endamœba coli*, and the two parasites are frequently confused, as it is often impossible to differentiate them at this time.

The pre-cystic forms are not present in the stools during the acute symptoms of dysentery but begin to appear as the symptoms subside, and during convalescence, when the stools are semi-formed or almost formed. I have observed them in small numbers during an acute attack on days in which the diarrhœal symptoms had disappeared and the stools were of a mushy consistence, but on the return of the diarrhœa they promptly disappeared and only the motile trophozoites could be found.

**3. Cystic Stage.**—*a. Unstained Preparations.* The cysts of *Endamœba histolytica*, in the living condition, appear as colorless, hyaline bodies, having a double outline when properly in focus, due to the cyst wall. They vary in shape but are, in the vast majority of instances, perfectly round. Oval and irregularly shaped cysts may sometimes be observed, but I believe that such forms are abnormal, and due to some unnatural condition. The cyst wall consists of a layer of refractile material which has a double outline and is always present. Forms are often observed which apparently have no cyst wall, but careful examination will demonstrate that such forms are really pre-cystic amœbæ, for in many instances, both pre-cystic forms and cysts are numerous in the same sample of fæces.

The size of the cysts varies greatly and races have been demonstrated which invariably produce cysts of either large or small size indefinitely. The size of the unstained cysts varies from 5 to 20 microns in diameter, and, rarely, cysts may be observed measuring more than 20 microns in diameter, but such large forms are very rarely observed.

The nuclei are poorly differentiated in unstained preparations but may sometimes be distinguished within the cyst as minute ring-like bodies composed of refractile granules or as refractile round masses in which the granular structure is not visible. A large vacuole is present in some



of the cysts, while others present refractile oval, bar- or rod-like masses, the chromidial bodies, but these are frequently invisible in the unstained cysts. The cytoplasm of the cysts does not contain any ingested material, as red blood corpuscles, as such material has been eliminated during the pre-cystic stage of development.

*b. Stained Preparations.* For the best results in the study of the morphology of the cysts specimens should be wet-fixed and stained with hæmatoxylin, but good results, from a diagnostic standpoint, may be obtained with the iodine stain, which brings the nuclei into view so that they can be counted. This stain will be discussed in the section treating of the diagnosis of *Endamæba histolytica* and the morphology of the cysts as stained with hæmatoxylin will be here described.

The cytoplasm of the cysts stains a grayish-brown, while the nuclear structures stain black. The cyst wall does not stain and is visible as a hyaline border surrounding the cyst. The chromidial bodies stain black and are very prominent when present.

The size of the cysts in stained specimens varies greatly, and it is now well established that *Endamæba histolytica* is divided into races distinguished by the size of the cysts that they produce. The first observer to describe such races was Ujihara (1914), who described five races having cysts of  $6.8\mu$ ,  $8.5\mu$ ,  $10.24\mu$ ,  $11.95\mu$ , and  $13.6\mu$ , mean diameter. Wenyon and O'Connor (1917) and Dobell and Jepps (1917-18) carefully studied the cysts of this amœba, and the latter authors described five races having cysts of the mean diameter of  $6.6\mu$ ,  $8.3\mu$ ,  $11.6\mu$ ,  $13.3\mu$ , and  $15\mu$ . Their observations have been confirmed by those of others, although all investigators do not accept the existence of as many races as do Dobell and Jepps, and a few observers, as Mathis and Mercier (1917), do not admit the existence of races producing different sized cysts.

Boeck (1923) divides the races of *Endamæba histolytica* into three groups: a small race, having cysts from 6 to 9 microns in diameter; a medium race, having cysts from 10 to 12 microns in diameter; and a large race, having cysts from 13 to 15 microns in diameter. For practical purposes I think that this classification is to be preferred to that of Dobell and Jepps, although there is no doubt of the existence of the races described by the latter authors, for I have personally observed the variations in the size of the cysts noted by them.

Dobell and Jepps (1918) have shown that races producing both large and small cysts may exist at the same time in the intestine, and the two varieties of cysts may be demonstrated in the stools simultaneously.

The shape of the cysts of *Endamæba histolytica* in stained preparations is generally round, but slightly oval cysts may sometimes be observed. The bizarre forms which Dobell (1919) states that he has seen I have



never observed, although I have seen degenerated cysts that were more or less irregular in shape. The vast majority of the cysts of this species are round in shape.

The cytoplasm in hæmatoxylin-stained specimens appears granular and reticulated, but vacuoles are not present unless the amœbæ are degenerating. The cytoplasm contains the nucleus and the chromidial bodies which are characteristic of this species.

The nucleus presents the same structure as the nucleus of the trophozoite, having a very delicate nuclear membrane and a very small, central karyosome. Upon the inner surface of the nuclear membrane minute grains of chromatin are collected, but there is not the regularity in their arrangement that there is upon the membrane of the nucleus of the trophozoite. In many of the nuclei there is a concentration of the chromatin at one pole of the nucleus and the nuclear membrane in this region appears thickened. The size of the nuclei varies with their number, the nucleus in the uninucleate cysts being large, its diameter being at least one-fifth of that of the cyst, while in the quadrinucleate cyst, the nuclei are small, their diameter not exceeding more than one-sixth of that of the cyst and often less. The cyst, when fully developed, contains four nuclei, but cysts are seen containing one, two, or four nuclei and atypical cysts are observed containing three nuclei.

The nuclei contain a very small *karyosome* appearing as an intensely staining black dot, generally situated at the centre of the nucleus but sometimes slightly displaced to one side of the centre. In uninucleate cysts the karyosome is sometimes surrounded by a ring of black chromatic granules and traces of a linin net-work may be observed extending from these granules.

A large *vacuole*, the glycogen vacuole, is frequently present in the uninucleate and binucleate cysts, sometimes so large as to almost fill the cyst, and to crowd the nuclei to one side. This vacuole apparently disappears as the cyst matures, for it is not found in the four-nucleated cyst except in rare instances.

*Chromidial bodies*, which stain black, occur in at least 50 per cent. of all cysts of *Endamæba histolytica*. These bodies are most numerous in the uninucleate and binucleate cysts and are best studied in freshly voided cysts, as they disappear from the cysts after the fæces containing the latter have been kept for a few days. This observation was first made by Dobell and I can confirm it from personal experience in the study of the cysts.

The chromidial bodies, in stained specimens, are bar-, rod-, oval-, or spindle-shaped, with rounded ends, and stain very dark brown or black. In some of the smaller cysts these bodies may be in the form of thin

rods resembling crystals, but the ends are always rounded and they can be easily distinguished from the chromidial bodies in the cysts of *Endamæba coli*.

The cytoplasm of the cysts in stained specimens never shows any ingested material.

*Supernucleate Cysts.* The fully developed cysts of *Endamæba histolytica* contain four nuclei. Several authorities have described cysts of this species containing eight nuclei. Kuenen and Swellengrebel (1913), Swellengrebel and Schiess (1917), and Brug (1917) all claim to have observed cysts of this species containing eight nuclei, but the possibility of a mixed infection with *Endamæba coli* was not eliminated. Dobell (1919) states that he has examined hundreds of thousands of

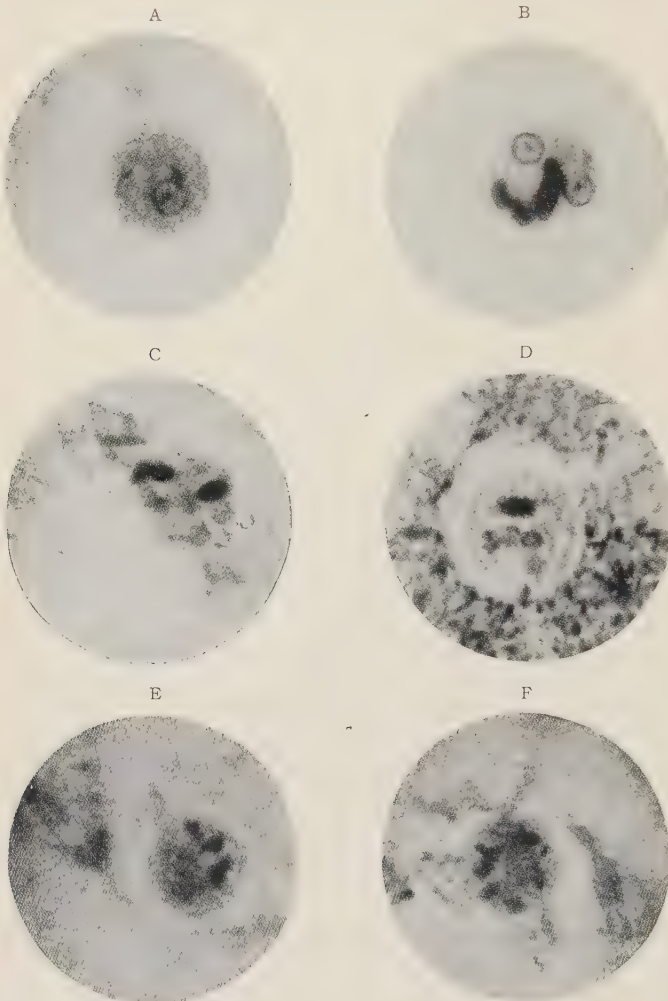


FIG. 5.—*Endamæba histolytica*. Cystic forms. (Photomicrographs. From Army Medical School Collection.) Stained with iron-hæmatoxylin. A. *E. histolytica* just at the time of encystment, containing one nucleus similar in structure to that in Fig. 4H.  $\times 1,150$ . B. Encysted form of *E. histolytica*, showing the primary division of the nucleus into two daughter nuclei.  $\times 1,150$ . C. Encysted form of *E. histolytica*, showing three daughter nuclei. The fourth nucleus, if ever present, has degenerated and is not visible.  $\times 1,150$ . D. Encysted form of *E. histolytica*, showing four daughter nuclei. This is the fully developed form of the cyst and is typical of this species.  $\times 1,150$ . E and F. Degenerated cystic forms of *E. histolytica*, showing the distribution of the chromatin in masses in the cytoplasm. These forms have also been misinterpreted as "budding" forms or reproduction by spore formation, by some authorities.  $\times 1,150$ .

cysts of *Endamæba histolytica*, and has seen only three cysts that might have been eight-nucleated cysts of this species, but of this he could not be

sure owing to the difficulty of excluding a mixed infection with *Endamœba coli*. However, he believes that such cysts probably occur, as supernucleate cysts are present in other species of parasitic amœbæ, and it is not probable that this species is an exception to so general a rule. In an experience covering many years in the study of *Endamœba histolytica*, during which time I have examined cysts from hundreds of cases of infection with this parasite, I have never observed a cyst of this species containing more than four nuclei, and I am very doubtful that such cysts ever occur. If they do, it must be so rarely that the chances of observing them are very slight and their occurrence is certainly of no practical importance from the standpoint of diagnosis.

**Resistance of Cysts.**—The resistance of the cysts of *Endamœba histolytica* to physical and chemical agents is a subject of great importance in the prophylaxis of infection with this parasite, for the cysts are the infective agents, and their destruction is, therefore, of primary importance in prophylaxis. Until quite recently very little was known regarding this subject, but a considerable amount of valuable data has been accumulated which can be applied in the prophylaxis of this parasite.

*Survival in the Fæces.* If the fæces containing the cysts of *Endamœba histolytica* are kept in a moist condition at room temperature or slightly below, the cysts will remain alive for several weeks. Thompson and Thompson (1916) found some of the cysts unchanged in moist fæces after one month, but the vast majority of the cysts perish by the end of the third week.

*Survival in Water.* If the fæces be greatly diluted with water the cysts survive for several weeks. Wenyon and O'Connor (1917) found that they remained viable for over a month, and Thompson and Thompson (1916) found them unchanged after twenty-five days. Dobell (1919) found cysts alive that had been kept in fæces in water for five weeks. The observations prove that the cysts of this species will remain alive in fæces greatly diluted with water for a period of from three to five weeks.

If the cysts are removed from the fæces by filtration and repeated washings and then placed in distilled water they will remain alive for a long period. Boeck (1921) found that cysts of this species of amœba thus treated and kept at a temperature between 12° and 22° C. (53.6° and 71.6° F.) remained alive for as long as 153 days. In running water, Penfold, Woodcock, and Drew (1916) found that the cysts would remain viable for fifteen days.

Kuenen and Swellengrebel (1913) record some experiments which show the effect of temperature and of bacterial growth in water containing the cysts. They found that at temperatures between 27° and 30° C. (80.6° and 86° F.) when there was a rich bacterial growth in the water the cysts were all dead after nine days, and if the temperature was

kept at 37° C. (98.6° F.) they all died within three days. On the other hand, if the water contained little bacterial growth and the mixture of cysts and water was kept at room temperature, some of the cysts remained viable for twenty-nine days.

Boeck (1921) found that if the cysts were washed and then placed in distilled water and the preparations sealed with vaseline and kept at temperatures between 12° and 22° C. (53.6° and 71.6° F.), some of the cysts remained viable for 211 days.

*Resistance to Desiccation.* The cysts of *Endamæba histolytica*, unlike those of the free-living amœbæ, do not resist drying for any length of time. Kuenen and Swellengrebel (1913) found that they all died in a few minutes when dried in the air, and Wenyon and O'Connor (1917) found that drying kills the cysts instantly. This fact demonstrates the impossibility of the transmission of the infection to man through dust, a theory which has been exploited by some epidemiologists, especially in tropical and subtropical regions, where drying occurs very quickly.

*Thermal Death Point of Cysts.* In a series of very careful experiments, Boeck (1921) has determined that the thermal death point of the washed cysts of *Endamæba histolytica* is 68° C. (154.4° F.), a temperature which some of the cysts withstand for five minutes.

*Resistance to Chemicals.* The data regarding the resistance of the cysts of this species of amœba to chemical agents are not very extensive but some practical points have been brought out in researches upon the subject. The chemicals that have been reported upon are bichloride of mercury, cresol, formalin, and chlorine.

*Bichloride of Mercury.* Kuenen and Swellengrebel (1913) found that a solution of bichloride of mercury, 1-1000, killed all of the cysts after an exposure of four hours.

*Cresol.* The same investigators found that a 1-250 solution of cresol in water killed the majority of the cysts in from five to ten minutes. Wenyon and O'Connor (1917) determined that a 1-20 solution of cresol killed the cysts practically instantly and that a 1-250 solution killed all of the cysts in fifteen minutes.

*Formalin.* This has not been found to be an efficient agent for the destruction of the cysts of this amœba. Kuenen and Swellengrebel (1913) state that they did not find any dead cysts after a few moments' exposure to a 10 per cent. solution of formalin, and Boeck (1921) found that some of the cysts remained viable after five days' exposure to a 5 per cent. solution of formalin.

*Chlorinated Water.* The question of the resistance of the cysts of *Endamæba histolytica* to chlorinated water is a most important one from the standpoint of the prevention of infection with this parasite, as the chlorination of water is now so largely depended upon for the preven-



tion of infections through water supply either in civil life or in campaigns. Unfortunately, the cysts of this amœba are not affected by the amount of chlorine used for the sterilization of water, as Wenyon and O'Connor (1917) have shown beyond question that very strong solutions of chlorine have no effect upon the cysts. They state: "Free chlorine in water to a strength of 1-10,000 has no effect on the cysts even after several hours' exposure." As the strength of chlorine mentioned is 100 times that usually used in the sterilization of water supplies it is evident that the chlorination of water as practised is no protection against infection with the cysts of *Endamœba histolytica*, and it would be impossible to use sufficient chlorine in water to accomplish sterilization so far as these cysts are concerned.<sup>1</sup>

*Survival in and upon Flies.* It has been definitely proven that the cysts of *Endamœba histolytica* can pass uninjured through the intestine of flies and that transmission of the infection may be caused through contamination of food by the droppings of these insects.

The cysts that adhere to the bodies of flies when feeding upon infected material die rapidly from desiccation, but if the insect walks over moist food quickly after feeding the cysts may be thus transmitted.

Thompson and Thompson (1916) were the first to call attention to the fact that flies feeding upon the cysts of this amœba contained the cysts in their alimentary tract, and that the cysts could also be found in their dejecta. Wenyon and O'Connor (1917) found that cysts of this amœba remained alive in the intestine of the fly for as long as twenty-four hours, and that living cysts were deposited in the insect's fæces for at least sixteen hours after feeding. They also found the cysts of this amœba in flies that were caught wild. Roubaud (1918) found that the cysts of *Endamœba histolytica* passed unchanged through the intestine of the fly and were voided in a viable condition for twenty-four hours, and, rarely, for forty hours after ingestion by the fly. Root (1921) found that the trophozoites of *Endamœba histolytica* when ingested by flies were killed in an hour or less in the intestine and never encysted. The cysts lived for a considerable time, about half of the cysts being dead after fifteen hours, the last living cysts being observed after forty-nine hours. In flies that were drowned in water the cysts were found alive in the insect's intestine for as long as seven days, which confirmed Roubaud's observations made in 1918.

It may be stated that the cysts of *Endamœba histolytica* remain viable in the intestine of the fly for a period of at least twenty-four hours, in the majority of instances, and that during this time the fæces of the insect contain the viable cysts.

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<sup>1</sup> Bercovitz (1922) has shown that chlorinated lime has no effect upon the cysts of *E. histolytica* in concentrations of 1 per cent.

*Standard of Viability of Cysts.* The determination of the viability of the cysts employed in most of the experiments mentioned above, and which has been proven to be accurate, is the reaction of the cysts to eosin solution. It has been demonstrated that the living cyst of the parasitic amoebæ and flagellates is not stained with eosin but that, when death occurs, the cysts stain with the eosin. A solution of neutral red has been found by Root (1921) to act in the same way, and either solution may be used in the determination of the resistance of the cysts to various agents with excellent results.

**Habitat.**—The natural habitat of *Endamoeba histolytica* is in the tissues of the large intestine of man, especially the region adjacent to the ileocæcal valve and in the rectum. The vast majority of cases of amoebic dysentery that come to autopsy show the most extensive ulcerations in these regions, while the remainder of the large intestine may be free from ulcers or very slightly affected.

Although this species of amoeba normally lives in the tissues of the large intestine, it can exist in those of the small intestine, as I have observed several cases in which typical amoebic ulcerations containing the amoebæ were present in the lower portion of the ileum in the region just above the ileocæcal valve. In the intestine the amoebæ live in the mucous and submucous coats, and, in many instances, in the muscular coat of the intestine. In these locations the amoebæ produce the very characteristic ulcerations of amoebic dysentery, but in only a very small proportion of infected individuals are the ulcers numerous or extensive enough to produce symptoms. In most individuals, while destruction of the tissue invaded by the parasites occurs, the lesions are slight and quickly heal, no definite symptoms of the infection being present.

*Endamoeba histolytica* has been found in many other locations in the human body, but its occurrence in these locations is purely accidental. It has been found in abscesses of the liver, spleen, and lung, and in the tissues of the bladder, testis, and epididymis. Recently Kofoed and Swezy (1922) and Ely, Reed, and Wyckoff (1922) have recorded the presence of this parasite in the bone lesions of the non-bacterial type of arthritis deformans, and Kofoed, Boyers, and Swezy (1922) have found it in the glands in Hodgkin's disease. These observations still await confirmation.

*Endamoeba histolytica* has also been reported as present in the blood and in lesions of the skin, but it is very doubtful if the observations upon which these reports were based were accurate. The amoeba has been reported in the urine in several cases and there is no doubt that, under certain conditions, it may be found in this situation. I have observed an instance in which a fistula had formed between an ulceration in an adherent portion of the intestine and the bladder, and the amoebæ

were frequently discharged into the urine through this opening and were demonstrated in the urine.

The amœbæ occurring in abscesses of the liver and lung, and also in other locations in the body outside of the intestine, are identical in morphology with the motile trophozoites found in the tissues of the intestine, but I have never observed cysts of *Endamœba histolytica* in these locations. It is evident that encystation cannot occur elsewhere than in the intestine and, therefore, that the normal life-cycle of this parasite cannot be maintained outside of the intestine, and that the location of the parasite elsewhere is abnormal and eventually fatal to its continued existence.

So far as is known, *Endamœba histolytica* does not live in the body of any of the lower animals under natural conditions.

**Species Occurring in Lower Animals.**—There is no evidence that demonstrates that *Endamœba histolytica* is a parasite of any of the lower animals, although some of the latter are infected with endamœbæ that so closely resemble it in morphology as to be practically indistinguishable from it. The following is a list of the most important endamœbæ which are parasitic in lower animals and their hosts:

- Endamœba aulastomi*, Noller, 1912. Host, leech.
- E. barreti*, Taliaferro and Holmes, 1924. Host, turtle.
- E. blattæ*, Leidy, 1879. Host, cockroach.
- E. bovis*, Liebetanz, 1905. Host, cattle.
- E. cobayæ*, Walker, 1908. Host, guinea-pig.
- E. intestinalis*, Walker, 1908. Host, cat.
- E. lacertæ*, Hartmann, 1907. Host, lizard.
- E. lagopidis*, Fantham, 1910. Host, moor-hen.
- E. muris*, Grassi, 1881. Host, mouse and rat.
- E. nuttali*, Castellani, 1908. Host, monkey.
- E. pitheci*, v. Prowazek, 1912. Host, monkey.
- E. polecki*, v. Prowazek, 1912. Host, swine.
- E. ranarum*, Grassi, 1879. Host, frog.
- E. salpæ*, Alexeieff, 1912. Host, fish.
- E. suis*, Hartmann, 1910. Host, swine.
- E. testudinis*, Hartmann, 1910. Host, turtle.

Of the species mentioned above, *Endamœba ranarum*, of frogs and toads; *Endamœba aulastomi*, of the leech; and *Endamœba nuttali*, of monkeys, are morphologically practically identical with *Endamœba histolytica*, and there is no evidence at present that they are not identical. However, Dobell has shown that it is impossible to infect tadpoles with the cysts of *Endamœba histolytica*, which would indicate that it is not identical with *Endamœba ranarum*.

Several authorities have found amœbæ in the stools of monkeys suffering from dysentery, and several species have been described and named. Castellani (1908) found an amœba in a spontaneous liver abscess in a monkey in Ceylon and named it *Endamœba nuttali*, while Mathis (1913)

described amœbic cysts containing four nuclei occurring in the intestine of monkeys in Tonkin and named the species *Löschia duboscqi*. The same species was apparently redescribed by v. Prowazek, in 1912, who named it *Entamœba pitheci*, and by Swellengrebel, in 1914, who named it *Entamœba chattoni*. In 1915, Macfie described an amœba in a monkey on the Gold Coast, and named it *Entamœba cercopithecii*. Dobell (1919), after a careful review of all of the evidence concerning these species in monkeys, states that they are all probably identical, at least in part, with *Endamœba nuttali*, Castellani, 1908, and that this species is at present indistinguishable from *Endamœba histolytica*.

**Cultivation.**—Many investigators have claimed to have cultivated *Endamœba histolytica*, but it is only recently that this species has been cultivated. Most of the cultures of amœbæ that have been obtained from dysenteric fæces were undoubtedly of free-living species, which were confused with the parasitic amœbæ. This mistake was made by Kartulis (1890), Musgrave and Clegg (1904), Walker (1908), Williams (1911), and others, and it is now generally accepted that none of the amœbæ cultivated by these authors were parasitic in nature, but were free-living species that had contaminated the material cultured or the culture media. Walker (1911), later, as the result of his investigations of cultural amœbæ, concluded that all of the amœbæ cultivable from the intestinal tract of man or animals were free-living species having nothing whatever in common with the true parasitic species. His work confirmed my own regarding this matter, and has since been confirmed by numerous observers. It is well known that the cysts of the free-living amœbæ are very resistant to drying and are carried about by the wind, thus reaching food and drink. These cysts when ingested by man pass through the intestinal tract unchanged and may be easily cultivated from the fæces upon simple culture media. In this way the amœbæ were apparently cultivated from the intestine of man, but a careful study of the morphology of these cultivated amœbæ should have been sufficient to distinguish them from *Endamœba histolytica* or the other parasitic amœbæ of man. In many instances the fæces were undoubtedly contaminated directly by the cysts of the free-living species carried in dust, as these cysts retain their vitality for weeks in dust. It may, therefore, be stated that at the present time the reports of the cultivation of the parasitic amœbæ, published prior to 1918, have all been proven to be based upon erroneous observations, and that all the amœbæ cultivated belonged to free-living species belonging to the genus *Amœba*.

In 1918, Cutler claimed to have cultivated *Endamœba histolytica* upon a blood-clot medium and upon an egg medium. The cultures were found to grow best at temperatures between 28° and 30° C. Cutler obtained



cultures from six stools from acute dysentery cases, and found that the cultures in one case could be kept going indefinitely. Daily subculture gave the best results, but if the medium did not become acid, subcultures were successful if made every two or three days. This paper of Cutler deserves more consideration than it has obtained, and his work should be carefully repeated by other observers, as he was apparently dealing with *Endamæba histolytica*. Dobell (1919) was not able to confirm his results, and evidently does not believe that he cultivated *Endamæba histolytica*, but the subject is one that demands more careful consideration before it can be said that Cutler's results were erroneous.

Yoshida (1919) claims to have cultivated *Endamæba histolytica* upon a medium composed of two parts of 2 per cent. sugar and one part of defibrinated horse-blood, but his descriptions of the cultured amœbæ throw grave doubt upon his results. It is probable that he succeeded in cultivating only free-living species.

The recent work of Boeck (1924) and of Boeck and Drbohlav (1924-25) has proved conclusively that *Endamæba histolytica* can be cultivated, and that cultures of this parasite are capable of producing typical amœbic dysentery in kittens accompanied by the characteristic lesions observed in man. These investigators cultivated this amœba from two patients with amœbic dysentery whose stools were free from other intestinal protozoa. The first successful culture was obtained upon tubes of Locke's egg medium while cultivating the stools of one of the patients for other intestinal protozoa. This strain of the parasite was continued in cultures for over eight months and through 152 subcultures. The second strain was obtained from the stools of a second patient, was subcultured for three months, and was then accidentally lost.

The media used by Boeck and Drbohlav are described in the Appendix. The morphology of the amœba in the cultures is identical with that observed in the stools in man; the cultivated amœbæ ingested red blood corpuscles; and typical cysts were observed in the cultures. The pathogenicity of both strains was proved by rectal injections of the cultures into kittens, and it was found that cultivation did not affect the virulence of the amœbæ. The lesions produced in the intestines of the kittens were typical amœbic ulcerations, and the amœbæ were found in the ulcers and recovered by cultivation. In one of the kittens a typical hepatic abscess appeared containing *Endamæba histolytica*.

In a personal communication Boeck (1925) informs me that this work has been repeated by Drbohlav, in both London and Paris, and that cultures of *Endamæba histolytica*, isolated by him, are now being carried along by Dobell and Wenyon, who have accepted this work. It is, therefore, certain that these observers have conclusively demonstrated that this parasite can be cultivated, and that the cultures are pathogenic to kittens, and I

believe that the credit for first undoubtedly cultivating this amœba belongs to Boeck and Drbohlav, for the work of Cutler was never confirmed by any protozoologist, while that of Boeck and Drbohlav has been amply confirmed by other protozoologists.

In this connection it should be mentioned that Barret and Smith (1923) cultivated a parasitic amœba from the turtle, which was later named *Endamœba barreti*, by Taliaferro and Holmes. This species is probably identical with *Endamœba testudinis* Hartmann, 1910, for in the cultures that I have examined the amœbæ are morphologically indistinguishable from the latter species, so far as it is possible to judge from Hartmann's description. This amœba is easily cultivated, and can be subcultured for many generations upon proper culture media.

**Life-history.**—So far as it is known the life-history or cycle of *Endamœba histolytica* is very simple. When the cysts of this species are ingested by man they pass through the stomach unchanged but when they reach the intestine the cyst wall is dissolved and the young amœba or amœbæ are liberated. It is still undecided as to what portion of the intestinal tract is selected for the liberation of the amœbæ from the cysts. Chatton (1917) apparently proved that excystation occurred in the small intestine, while Izar (1914) found that he could not infect kittens by rectal injection of material containing only cysts of this parasite. Recently, Sellards and Theiler (1924) have shown that it is possible to infect kittens by injecting material containing only cysts of *Endamœba histolytica per rectum* provided a condition of stasis be first produced in the animals, and they believe that excystation occurs in the large intestine, as their experiments show that it is possible. However, it is generally believed that excystation occurs in the small intestine, and the fact that I have observed several cases of amœbic dysentery in which ulcerations were present in the lower portion of the ileum gives support to this view.

The number of amœbæ liberated from the cysts at the time of excystation is also still undetermined. Chatton (1917) states that only one multinucleate amœba is liberated from the cyst containing four nuclei, and that these nuclei merge into one nucleus later, but his observations have not been confirmed. Dobell (1919) leans to the view that four amœbæ are liberated from the cyst, and it is certainly more probable that this is the fact, although there is no evidence available proving that such is the case.

After liberation the young amœbæ attack the epithelium of the large intestine, and by means of the cytolytic ferment which they secrete, they penetrate the tissue and cause the ulcerations characteristic of amœbic dysentery. Rarely they attack the mucous membrane of the lower portion of the small intestine, as evidenced by the presence of the ulcerations

and amœbæ in the lower portion of the ileum. The tissue-invading forms reproduce in the tissue of the intestine by fission into two organisms. The process of multiple fission, or schizogony, and of reproduction by gemmation or budding, described for this species by Schaudinn (1903), have been proven to be non-existent.

Certain of the amœbæ are continually present in the lumen of the bowel. These are smaller than the vegetative, or tissue-invading forms, and are known as pre-cystic amœbæ. The pre-cystic forms eventually become cysts, which, when fully developed, contain four nuclei. The cysts are voided from the intestine in the fæces and undergo no further development until ingested by man, when the process of excystation, tissue invasion, and formation of pre-cystic and cystic forms is again repeated. The cysts are at first uninucleate, but the nucleus divides into two daughter nuclei and these again into two, thus producing the four-nucleate cysts which alone are infective, in all probability.

Conjugation has not been demonstrated in *Endamœba histolytica* but I have several times observed phenomena which were most suggestive of this process and difficult of interpretation in any other way. Dobell (1919) thinks that conjugation may occur between the young amœbæ liberated from the cysts, although there is no evidence of such a process.

The formation of cysts does not occur when *Endamœba histolytica* invades other tissues than those of the intestine. Cysts have never been demonstrated in the abscesses of the liver, brain, or other organs in which this parasite has been found, so that it is certain that the normal life-cycle of this parasite occurs only in the intestine, and the presence of the parasite in other locations is purely accidental.

**Method of Reproduction.**—In the vegetative stage the trophozoites of *Endamœba histolytica* reproduce by simple fission, the binary division of the nucleus being followed by that of the body of the amœba. Reproduction in the cystic stage occurs by the division of the nucleus into two nuclei and these again into two, four nuclei being eventually present in the cyst.

There is still considerable disagreement among authorities as to whether the division of the nucleus is mitotic or amitotic in character, or intermediate in type. Schaudinn (1903) stated that division was amitotic, but his observations are now discredited. Dobell (1919) studied dividing amœbæ of this species in sections of the intestine of infected cats, a method which he claims is the only one that gives accurate results in the study of the division of the nucleus. Briefly summarized, his observations showed that prior to division the nucleus increased in size and the karyosome fragmented. The chromatin upon the nuclear membrane then moved toward the centre of the nucleus and the nucleus became oval in shape. Achromatic threads now appeared within the nucleus



and eventually it became spindle-shaped, and appearances suggestive of mitotic figures and chromosomes were observed. However, Dobell states that he has not been able to trace true mitosis in such amœbæ or to demonstrate the presence of chromosomes. The achromatic threads pass from one end of the spindle to the other, and are generally well defined, but vary in number and arrangement.

The spindle-shaped nucleus now becomes constricted in the middle, and eventually divides into two parts which may be connected by a slender thread for some time. After nuclear division is complete the two nuclei assume the structure of the nucleus of the trophozoite, the chromatin collecting upon the inner surface of the nuclear membrane, and a small, central karyosome developing. The entire organism now becomes constricted in the middle and eventually division occurs into two amœbæ. Dobell regards the division of the nucleus in this species as intermediate in type between amitosis and mitosis, so far as can be gathered from his description. The type of division of the nuclei in the cysts is similar, according to this author.

Kofoed and Swezy (1922) state that the division of the nucleus in *Endamæba histolytica* is mitotic in character, and that chromosomes are definitely present. The number of chromosomes they have not been able, as yet, to determine, but they state that it is small, not exceeding six.

My own observations regarding the method of nuclear division in *Endamæba histolytica* agree very largely with those of Dobell. I have not been able, in the preparations that I have studied, to distinguish definite chromosomes, although masses of chromatin occur within the dividing nucleus that might be easily interpreted as chromosomes by one predisposed to such an interpretation. However, the variation in number and arrangement of these little masses of chromatin appears to me to speak decisively against their being true chromosomes.

Binucleated amœbæ of this species are not so very infrequently found in the fæces. Dobell (1919) does not consider that these are normal dividing forms, but some of them are certainly indistinguishable from forms observed in sections of the tissues in which division of the nucleus has been completed.

**Geographical Distribution.**—For many years it was believed that the geographical distribution of *Endamæba histolytica* was confined to tropical and subtropical regions. This belief was largely due to the fact that the acute and severe symptoms caused by this parasite and collectively known as amœbic dysentery were much more frequently observed in patients in the tropics and subtropics than elsewhere, but the observations of recent years have demonstrated that the distribution of this species is practically world-wide, although amœbic dysentery is much more common in some regions, as the tropics, than in others. We now know



that the vast majority of individuals who harbor *Endamæba histolytica* present no symptoms of the infection, or the symptoms are so slight and atypical as to be overlooked, and that a goodly percentage of apparently healthy individuals in almost every locality is infected with this parasite.

**Incidence of Infection.**—Walker and Sellards (1913) were the first to demonstrate that *Endamæba histolytica* occurred in a considerable proportion of healthy individuals as well as in those suffering and convalescent from dysentery. Their observations awakened much interest in the incidence of infection with this and other intestinal parasites, and during the World War many authorities carefully investigated the incidence of this species of amœbæ in soldiers serving in different war areas. These investigations consisted of the microscopical examination of the stools for the amœbæ, and the figures given by different authorities vary considerably, due largely to the number of examinations made in each case. Dobell (1917) found that a single microscopical examination of the stools would only develop about one-third of the actual infections, while three examinations would show between one-half and two-thirds of the actual number of infections. It, therefore, followed that those investigators who made many examinations of the stools from each case obtained much higher percentages of positive results than those who made only one or two examinations, and Dobell's findings have been confirmed by Boeck and Stiles (1924) and many others. The results obtained by several workers along this line which follow give a good picture of the incidence of infection with *Endamæba histolytica* in many localities.

Wenyon (1916) examined 556 British soldiers who were patients in hospitals from the Mediterranean area and found 10.8 per cent. infected; Carter, Mackinnon, Mathews, and Smith (1917) examined the stools of 4,068 dysenteric convalescents at Liverpool who came from the Mediterranean area and found 12.1 per cent. positive for *Endamæba histolytica*, the average number of examinations being three; Smith and Mathews (1917) examined 450 non-dysenteric convalescents in Liverpool and found 6.4 per cent. positive; MacAdam (1919) examined 946 men in India of whom 385 were convalescent from dysentery, and 351 were non-dysenteric patients. Of the dysentery convalescents 17.8 per cent. were positive and of the non-dysenteric patients, 13.6 per cent. were positive for *Endamæba histolytica*. In the same year, Turner and Turner (1919) examined 3,277 British soldiers and found 15.4 per cent. infected with this parasite. Most of these soldiers came from service in France, and the incidence in these men was 11.4 per cent. The remainder came from Salonica, Mesopotamia, and East Africa, and the incidence of infection in these was 24 per cent. Brug (1920) found that in Java, 21 per

cent. of Europeans, 16 per cent. of natives, and 17.5 per cent. of soldiers were infected with *Endamæba histolytica*.

Kofoid (1920) and his co-workers examined 2,300 American soldiers who had served overseas, mostly in France, and found 297 infected with this parasite, or 12.8 per cent. Of 576 home-service men they found only 25 infected, or 4.3 per cent., only one examination being made in these cases. Jepps (1921) examined 971 British soldiers in hospital at Southampton. Of these 527 were men suffering from, or convalescent from, dysentery, 210 were suffering from other intestinal complaints, and 95 from non-intestinal conditions. Of the 971 cases, 230, or 23.7 per cent., were positive for *Endamæba histolytica*; of the 527 suffering from, or convalescent from, dysentery, 26.5 per cent. were positive; of the 210 cases suffering from other intestinal conditions, 19.5 per cent. were positive; and of the 95 non-intestinal cases, 23.2 per cent. were positive. Of the latter cases, 25 gave a history of dysentery, and 32 per cent. were positive, while 70 gave no history of dysentery, and 20 per cent. of these were positive. The vast majority of these cases were examined six times (777 cases), while in only 50 cases was only one examination made. Fletcher and Jepps (1924) examined 1,034 Asiatics in the Federated Malay States and found 150, or 14.5 per cent., infected with *Endamæba histolytica*.

Faust and Wassell (1921) record the examination of 241 individuals at Wuchang, China. Of these, 57 were patients in the hospital, some suffering from dysentery; 46 were coolies; 40 were house servants of Europeans; and 98 were Europeans. Of these, 50.9 per cent. of patients were positive for *Endamæba histolytica*; 10.8 per cent. of coolies; 2.5 per cent. of the servants; and 27.5 per cent. of the Europeans. Dobell (1921) compiled the data accumulated by different observers as to the incidence of infection with *Endamæba histolytica* in natives of England who had not been abroad, and found that only 3.4 per cent. of 3,146 individuals examined were infected with this parasite.

In a series of examinations made upon students at the University of California, Kofoid and Swezy (1921) obtained very high percentages of infection, but the number of individuals examined was small, which, undoubtedly, partially accounts for their high results. They examined 154 students, of whom 91 served in the World War overseas, 34 were on home service, and 29 were not determined. Of the 91 overseas men, 61, or 67 per cent., were positive for *Endamæba histolytica*; of the 34 home-service men, 9 were positive, or 26.5 per cent.; and of the undetermined, 29 in number, 12 were positive, or 41.4 per cent. An average of 3.8 examinations was made in each of these individuals.

Probably the most important survey of the incidence of infection with *Endamæba histolytica* that has been made is that of Böeck (1923),

who examined 8,029 individuals in the United States. This survey covered four classes of persons, *i.e.*, United States soldiers having foreign service; United States soldiers with home service only; individuals with no military service; and those of undetermined status in this respect. Of the first class, 3,536 were examined; of the second, 2,584; of the third, 1,547; and of the fourth, 362. Of the total 8,029 individuals, 333, or 4.1 per cent., were infected with *Endamæba histolytica*, an average of 1.6 examinations being made *per capita*. In 505 individuals, six examinations were made *per capita* and *Endamæba histolytica* was found in 15 per cent., thus demonstrating that when only one examination is made *per capita*, only about one-third of the infections are found. Boeck was unable to confirm Kofoed's findings that infections with *Endamæba histolytica* were much more numerous in our soldiers who had foreign service than in the home-service men, as he found the rates practically the same. The rate in the men having foreign service was 2.8 per cent., as compared with a rate of 3.5 per cent. in the home-service class. In the no-service class Boeck found 8.3 per cent. of infections and in the undetermined class, 3 per cent. of infections. The much higher percentage in the no-service class was due to the fact that included in this class were many individuals in institutional life, in whom the incidence of infection with this parasite, as well as others, is always high, due to poor sanitation and increased facilities for transmission through food handlers and contact.

Boeck believes that had six examinations per capita been made in the service class of cases, the incidence of infections would have been not larger than 10 per cent. In one institution he found the incidence of infection with this parasite to be as high as 27.3 per cent., six examinations being made per capita. He also demonstrated that the incidence of infection increased with the length of residence in the institution.

So far as the United States is concerned, I believe that it is safe to estimate that about 10 per cent. of the population is infected with *Endamæba histolytica*. This percentage will be much smaller in some localities than in others, but it is believed that this is a conservative estimate of the extent of infection in this country with this parasite.

**Method of Transmission.**—*Endamæba histolytica* is transmitted from man to man through food and drink contaminated with the cysts of the parasite. Only the cysts are infective to man, and man is only infected naturally through the ingestion of food and drink contaminated by the cysts. As already noted, the cysts remain alive in moist fæces for a considerable period of time, so that contamination of food and drink is easily possible in many ways. The fact that infection is possible only by the *ingestion* of the cysts places amœbic dysentery in the same class of infections with the typhoid fevers, bacillary dysentery, and cholera.



The cysts of *Endamæba histolytica* are the infective agents, and as these do not occur in the fæces of patients presenting the symptoms of acute dysentery, it follows that such individuals are not concerned in the transmission of the infection. Apparently healthy individuals, and those that are convalescent from an attack of amœbic dysentery, are the sources of infection, and these "carriers" are numerous in all regions where this type of dysentery occurs, especially in the tropics. To Walker (1911), and Walker and Sellards (1913), we owe the recognition of the vital importance of "carriers" in the transmission of *Endamæba histolytica*, and the fact that the parasite is transmitted naturally only through their agency.

Walker divided the "carriers" of *Endamæba histolytica* into two classes, "convalescent carriers," or those who had recovered from an attack of amœbic dysentery, and "contact carriers," or those who had never suffered from an attack of amœbic dysentery, but had acquired their infection through contact with an infected individual. Transmission of the parasite depends upon these "carriers," and as the vast majority of "carriers" are apparently healthy individuals the chances of transmission are very great when sanitary precautions are neglected.

Contamination of food and drink with the cysts of this parasite may occur through an infected water supply; through the use of human excreta in the fertilization of vegetables; through the handling of food by infected individuals; and through the droppings of flies. Other methods of contamination are conceivably possible, but those mentioned are the principal ones concerned in the transmission of the infection.

A contaminated water supply is probably the most common and important source of infection, for it has been shown that the cysts of *Endamæba histolytica* remain viable in water for several weeks. The severe outbreaks of amœbic dysentery among American soldiers in the field during the Philippine Insurrection were undoubtedly due to the constant use of badly contaminated water, and I have personally observed three cases of amœbic dysentery in which the infection was derived from drinking water from a water cooler that was cared for by a "carrier" of the infection.

The use of uncooked vegetables which have been fertilized with human excreta, a practice common in many tropical and subtropical countries, is a prolific source of infection with this parasite where this method of fertilization is employed, and I have personally observed several cases of amœbic dysentery in which the infection was acquired in this manner.

The handling of food and drink by "carriers" of the infection is almost invariably followed by the infection of those who ingest the food, owing to the certainty of its contamination with the cysts unless the most



Careful precautions are taken regarding the cleanliness of the hands of the food handlers. Cooks and mess attendants who harbor the cysts of this parasite are very efficient transmitters of this infection.

The contamination of food and drink by the fæces of flies that have fed upon material containing the cysts of *Endamæba histolytica* is undoubtedly a common, and important, source of infection, as the cysts pass through the intestine of the fly unchanged, and food and drink are contaminated by the insect's droppings. The cysts may remain viable in the intestine of the fly for a period of nearly two days, during which time the droppings of the insect contain the cysts, which are still viable, and thus contaminate food and drink.

In 1916, I observed a small epidemic of amœbic dysentery among soldiers of the United States army at El Paso, Texas, which was undoubtedly due to transmission by flies. This outbreak was very carefully investigated, and the transmission of the infection could not be explained in any other way. This epidemic is discussed later in the consideration of the relation of *Endamæba histolytica* to disease.

**Experimental Infection of Lower Animals.**—It has already been stated that many investigators have succeeded in infecting some of the lower animals with *Endamæba histolytica*. These infections have been produced either by the injection of the motile trophozoites into the rectum or by the feeding of material containing the cysts of the parasite.

The dog has been experimentally infected and amœbic dysentery produced by Lösch (1875), Hlava (1887), Harris (1901), and Dale and Dobell (1917). Typical amœbic ulcerations have been produced in these animals, and Harris (1901) observed abscess of the liver in dogs experimentally infected with this parasite.

The cat has been experimentally infected, with the production of amœbic dysentery, by numerous observers, the most extensive experiments being those of Marchoux (1899), Craig (1905), Wenyon (1912), and Dale and Dobell (1917). The lesions produced in these animals are quite typical of those present in the intestine of man in amœbic dysentery, and Marchoux (1899), Craig (1905), Wenyon (1917), and others have experimentally produced amœbic abscess of the liver in cats following the occurrence of amœbic dysentery in these animals. *Endamæba histolytica* does not encyst in the intestine of cats, a fact which explains why the cysts of this parasite were never observed by the early workers in the fæces of their experimental animals. Half-grown cats have been found to be the most susceptible to infection, and in these animals the rectal injection of motile trophozoites or the feeding of material containing cysts is followed by a severe and generally fatal dysenteric infection. In my personal experiments upon cats I found that the incubation period after rectal injection before the first symptoms of dysentery appeared

varied from six days to two weeks, while the period of incubation after feeding the cysts varied from seven to eleven days, the average period being about eight days.

Musgrave (1906) has produced dysentery in monkeys experimentally with *Endamæba histolytica*. There is no doubt of this, as I personally saw some of his experimental animals, and the lesions present, which were typical amœbic ulcerations in which *Endamæba histolytica* was present in large numbers. Unfortunately, Musgrave at that time did not recognize any differences between pathogenic and non-pathogenic species of amœbæ, and the organisms present were not identified by him as *Endamæba histolytica*.

Guinea-pigs have been experimentally infected with this species by Bætjer and Sellards (1914) and by Chatton (1918), while Huber (1909) was successful in infecting rabbits. The lesions produced by the parasite in these animals are quite different from those produced in dogs and cats, or in man, and symptoms of dysentery do not occur. The lesions in the intestine in guinea-pigs resemble neoplasms.

The rat has been experimentally infected with *Endamæba histolytica* by Lynch (1915) and by Brug (1919), and both of these investigators believe that this animal is a natural "carrier" of the infection, as these animals have been found naturally infected.

Amœbic dysentery sometimes occurs spontaneously in dogs and monkeys, and the amœbæ occurring in the intestine and in the fæces of these animals cannot be distinguished morphologically from *Endamæba histolytica*.

**Relation to Disease.**—All authorities are now convinced that *Endamæba histolytica* is a pathogenic parasite of man and the cause of a severe, and often fatal, disease known as amœbic dysentery. Prior to 1913, the evidence upon which this belief was based consisted of the characteristic pathology of the disease with which this parasite was always associated, while it was absent in other forms of dysentery; the experimental production of similar lesions in susceptible animals, as dogs and cats, by the rectal injection of the motile trophozoites, and feeding of the cysts of *Endamæba histolytica*; and the occurrence in both natural and experimental infections of a peculiar form of liver abscess, in the walls of which this species of amœba was invariably present. While this evidence of the etiological relationship of the parasite to the disease was sufficient to convince most observers, there were some who still doubted the pathogenicity of *Endamæba histolytica*, and it remained for Walker and Sellards, in 1913, to definitely demonstrate the etiological relationship of this parasite to disease. These investigators fed twenty volunteers, who were prisoners in Bilibid Prison, in Manila, with material containing the cysts of *Endamæba histolytica*, of whom eighteen

became parasitized, the amœbæ being found in their stools after incubation periods varying from one to forty-four days, the average period being nine days. Of the eighteen men who became parasitized, four, or 22.2 per cent., developed amœbic dysentery, the incubation period from the time of feeding to the development of symptoms being 20, 57, 87, and 95 days, with an average of 64.8 days.

Walker and Sellards observed the cysts of *Endamœba histolytica* in the stools of men who had been fed motile trophozoites, thus proving that such forms encysted in the intestine and were not destroyed by the gastric secretion, as generally supposed, but none of the men fed motile amœbæ developed dysentery.

All of the men who developed dysentery were fed cysts from the stools of healthy "carriers," and in two of the cases the disease followed the ingestion of cysts from the stools of "contact carriers" who had never had dysentery and did not subsequently develop the disease. In one experimental infection, 371 days and the passage through two "contact carriers" intervened between the case of natural and the case of experimental dysentery.

The above experiments demonstrate that *Endamœba histolytica* is the cause of amœbic dysentery in man, and that the cysts of this parasite occurring in the stools of healthy "carriers" are virulent and capable of causing the disease.

While dysenteric symptoms are the most serious evidence of infection with *Endamœba histolytica*, the vast majority of infections with this parasite are not accompanied by these symptoms, but by much milder symptoms, as indigestion, tenderness of the abdomen, slight diarrhœa, and general lack of vitality. Where one case of amœbic dysentery occurs there occur many cases of amœbic diarrhœa, characterized by short periods of mild diarrhœa alternating with periods of constipation, or evanescent digestive disturbances accompanied by more or less abdominal tenderness. It is my belief that most "carriers," who are generally held to be symptomless, in reality have symptoms of their infection, at some time, but these symptoms are not recognized by either the individual or the physician as due to infection with this parasite. While a history of dysentery is usually denied, a careful inquiry will elicit the information that slight attacks of diarrhœa have occurred, often at night, which subsided after the passage of one or more voluminous stools, while they will also admit that they suffer at times from discomfort after eating, intestinal indigestion, a feeling of malaise, and periods of loss of weight, especially in hot weather. All of these symptoms are often due to infection with *Endamœba histolytica*, and if carefully inquired for in individuals showing the cysts of this parasite in their stools, it will be found



that the majority of supposedly healthy "carriers" are not symptomless, but present definite clinical evidence of their infection.

*Endamæba histolytica* is a true tissue parasite, living upon the tissues of its host by cytolyzing the tissue cells and absorbing nutriment from the cytolyzed material. It therefore follows that there can be no infection with this parasite without the production of pathological lesions, however minute such lesions may be. Under ordinary conditions the lesions produced are so minute, and healing so quickly follows, that a condition of equilibrium is produced between the host and the parasite, and marked symptoms of infection are not present. This is the condition that obtains in the apparently healthy "carriers" of *Endamæba histolytica*.

It may be stated that there is no evidence that this species of amœba can exist in the intestine of man without producing lesions, and that all individuals who show cysts of the parasite in their stools must have lesions in the intestine. The presence or absence of clinical symptoms must depend either upon the virulence of the parasite or the resistance of the host to the infection, and these will now be discussed.

*Virulence of the Parasite.* Several authorities have claimed that different races of *Endamæba histolytica* vary in virulence and have endeavored to explain the severe symptoms caused by the parasite in some individuals and the absence of symptoms in others by this difference in virulence. Sellards and Bætjer (1915) claim to have observed three races of this parasite which differed experimentally in virulence, but their observations have not been confirmed.

There is no doubt that in certain regions, as the tropics, infections with this parasite are much more frequently followed by the symptoms of amœbic dysentery than in temperate regions, but that this is due to the greater virulence of the amœbæ in tropical regions is, to say the least, very doubtful. That virulence can have little, or nothing, to do with the production of symptoms, is proven by the fact that patients suffering from most severe amœbic dysentery in the tropics will rapidly improve and eventually recover if moved to a temperate region, and I have observed scores of individuals suffering from severe amœbic dysentery contracted in the Philippine Islands quickly recover when invalided home to the United States. To believe that the disappearance of symptoms in these cases was due to a lessened virulence in the amœbæ brought about by a change in climate is absurd, and the only conclusion that is justified is that the resistance of the individual to the infection has been increased by removal to a less enervating climate.

Another proof that there is no difference in the virulence of the amœbæ is furnished by Walker and Sellard's (1913) experiments in which amœbic dysentery was produced by the feeding of cysts to man,



the cysts being obtained from the stools of healthy "carriers" of *Endamæba histolytica*, who never had or ever developed symptoms of infection, thus proving that the cysts from these healthy individuals were just as capable of producing dysentery as those from the stools of patients who had suffered from the disease.

It may be stated that at present there is no evidence that races of *Endamæba histolytica* exist that vary in virulence, and it is most doubtful if such races ever occur.

*Resistance to Infection.* It is my belief, shared by many others who have had an extensive experience with amœbic dysentery, that the production of symptoms by *Endamæba histolytica* depends entirely upon the lack of resistance of the infected individual to the infection. The vast majority of infected individuals are so resistant that pronounced and definite symptoms of the infection never occur. It is only when this resistance is lowered by overwork, poor food, the enervating effect of a tropical climate, or constant heavy reinfections, that symptoms of dysentery develop. Any of these factors, operating separately or together, may serve to unbalance the equilibrium between parasite and host, and the host develops symptoms of the infection.

This fact was most perfectly illustrated in the instance of the United States troops serving in the Philippine Islands during the Philippine Insurrection. These troops were campaigning under the most trying conditions in a tropical country, constantly exposed to water supplies heavily contaminated with human excrement, frequently without facilities for procuring an adequate and suitable supply of food, and exposed to the rains and subsequent chilling characteristic of field service in that country. Under these conditions the resistance of the individual was greatly lowered, with the result that many of the men developed symptoms of amœbic dysentery, and hundreds were invalided to the United States from this cause. As soon as general conditions improved and active field service gave place to life in a properly sanitated post, amœbic dysentery gradually disappeared and ceased to be an important cause of morbidity in our troops in the Philippine Islands.

**The Epidemic Occurrence of Amœbic Dysentery.**—Some authorities, reasoning from purely biological premises, deny the possibility of the occurrence of epidemics of amœbic dysentery, just as certain zoologists used to deny the possibility of epidemics of the malarial fevers. Such an opinion will not be shared by any one who had experience with amœbic dysentery during the field service of our troops in the Philippine Islands during the Philippine Insurrection, for at that time veritable epidemics of this disease occurred among our troops. The argument that such epidemics were really due to bacilli of bacillary dysentery, used

by some recent writers, has no force, for these infections were studied by acknowledged authorities, as Flexner and Strong, and their amœbic origin established beyond question. Epidemics of bacillary dysentery also occurred at this time among our troops and were recognized bacteriologically, but there was no confusion between the two types of dysentery as observed in our large hospitals, and I personally know that the distinction between them was made microscopically and bacteriologically in all of our general hospitals. That epidemics of amœbic dysentery occurred among our troops at that time is a fact that admits of no contradiction in the minds of those who served through those trying days in the Philippines.

It is undoubtedly true that under the conditions prevailing in a modern town or city or in a properly sanitated military camp or post, an epidemic of amœbic dysentery is practically impossible, but under the conditions of active field service such epidemics will occur provided the troops are brought into contact with many "carriers" or with infected material. It must be admitted that the "dosage" of the infective principle must have much to do with the depletion of resistance of the infected individuals, and under the conditions that prevailed in the Philippines at that time, mass infection and consequent epidemics were inevitable. Grossly contaminated water supplies; the presence of flies that had fed upon the fæces of the heavily infected natives; a poor and often insufficient food supply; excessive physical exertion under a tropical sun; nervous strain and mental anxiety—all contributed to reduce the resistance of our troops, and scores and hundreds of cases of amœbic dysentery appeared within a comparatively short period of time. Under these conditions the incubation period was shortened, and thus many individuals came on sick report for this condition within a short time, thus causing an epidemic rise in the incidence of the disease.

In 1916, I observed a small epidemic of amœbic dysentery in our troops serving upon the Mexican Border. This epidemic has been described (1917), but will be briefly sketched here as it illustrates the possibility of an epidemic outbreak of this disease. From July, 1916, to November, 1916, 118 cases of amœbic dysentery were admitted to hospital in the person of soldiers serving in the El Paso District, and all but five of these cases originated in the camps in and around El Paso. The diagnosis in all cases was made by microscopic examination of the fæces and the demonstration of motile trophozoites of *Endamæba histolytica*, and in every case I personally examined the stools and confirmed the diagnosis. Cultures were negative for bacilli concerned in the etiology of bacillary dysentery, and the latter disease was not present among the troops in the El Paso District. This epidemic of amœbic

dysentery was traced to transmission by flies, which were very numerous in the camps at the time the disease occurred. No epidemic occurred in the civilian population of El Paso during this time although the water supply was identical with that used by the troops, and inquiry showed that in only eight cases had the men been absent on detached service at any time, so that the origin of the infection in the camps was unquestioned. The period of incubation in these cases varied from one week to six months from the time of arrival of the men in the infected district. Thirty-six per cent. of the cases developed within one month after arrival at camp, 66 per cent. within the first two months after arrival, and 90 per cent. within the first three months after arrival. This outbreak of amœbic dysentery demonstrates beyond question that the disease can occur in epidemic form when conditions are favorable.

**Pathology.**—*Endamœba histolytica* is naturally a parasite of the large intestine of man and primary infection occurs in the intestine. Secondary infections are, with the exception of abscess of the liver, very rare but have been reported as occurring in almost every organ of the body, but many of the reports are unreliable. The amœbæ reach the viscera through the blood or lymph streams.

The pathology of infection with this parasite will not be discussed here except briefly, and the reader is referred to the classical monograph of Councilman and Lafleur (1891) for a thorough discussion of this subject.

*The Intestinal Lesions.* The lesions of amœbic dysentery are most commonly observed in the rectum and in the area of the intestine just below the ileocæcal valve. Of 78 cases that I observed at autopsy in which the location of the lesions was especially noted, 57 showed lesions below the ileocæcal valve and in the rectum, the intervening portion of the intestine being uninvolved macroscopically; 12 cases showed lesions extending the entire length of the colon, but always most severe in the rectum and below the ileocæcal valve; while the remaining 9 showed lesions only in the rectum, and for a short distance above the sigmoid flexure. Lesions were observed in the ileum, just above the ileocæcal valve, in two cases.

The appearance of the ulcerated intestine in infections with this parasite has been described by many authorities and is well understood at the present time. Suffice it to state here that the lesions of this disease are absolutely characteristic and cannot be confused with those of bacillary dysentery by any one who has had autopsy experience with both infections. Some authorities have endeavored to prove that the lesions of amœbic dysentery do not differ markedly from those of the bacillary forms, but such statements can only be based upon a very limited experi-

ence, for even the tyro will not be confused if he has studied a few cases upon the autopsy table. Mixed infections sometimes occur, but the trained pathologist has no difficulty in recognizing them.

*The Liver.* Secondary invasion of the liver occurs through the portal vein, and a peculiar form of abscess is produced that is characteristic of this infection. The percentage of patients developing amœbic abscess of this organ varies considerably, and it is a much more frequent complication in the tropics than in the subtropics or temperate regions.

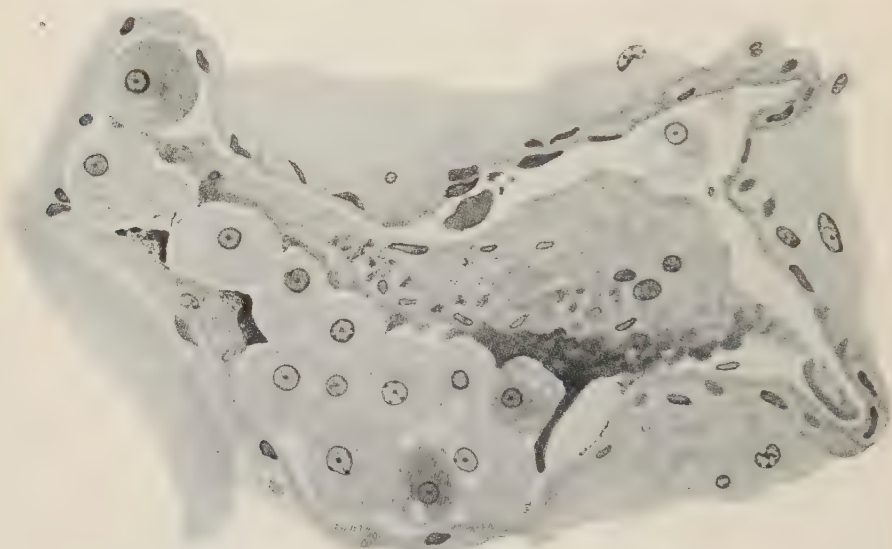


FIG. 6.—*Endamæba histolytica*. Cross-section of a small blood vessel in the margin of an intestinal ulcer showing numerous *Endamæba histolytica* within its lumen.  $\times 700$ . (After Kofoid and Swezy.)

Kartulis (1887) found 55 per cent. of 500 cases autopsied by him showed amœbic abscess, and Zancarol (1893) found this condition in 59 per cent. of 444 cases that came to autopsy, and in 78 fatal cases of the disease that I (1911) observed at autopsy, 33 per cent. showed abscess of the liver. However, it should be remembered that these statistics are based upon only the fatal amœbic infections observed and that the real percentage of patients suffering from this infection who develop abscess of the liver is very much smaller. Councilman and Lafleur observed only 21 cases of abscess of the liver in the 1,429 cases of amœbic dysentery that they studied, and in my experience this condition has not developed in over 5 per cent. of the cases that I have observed.

The abscesses may be single or multiple, and situated in any part of the liver. The most characteristic and most frequent condition is a single, large abscess situated in the right lobe, but multiple abscesses



are frequent and situated in both right and left lobes. The Lobus Spigelii is much less frequently involved.

When uncomplicated by a secondary infection with bacteria the abscesses of the liver produced by *Endamœba histolytica* are very characteristic. The abscess cavity is filled with semi-fluid, yellowish-red or chocolate-colored material, the cytolyzed liver tissue, composed of

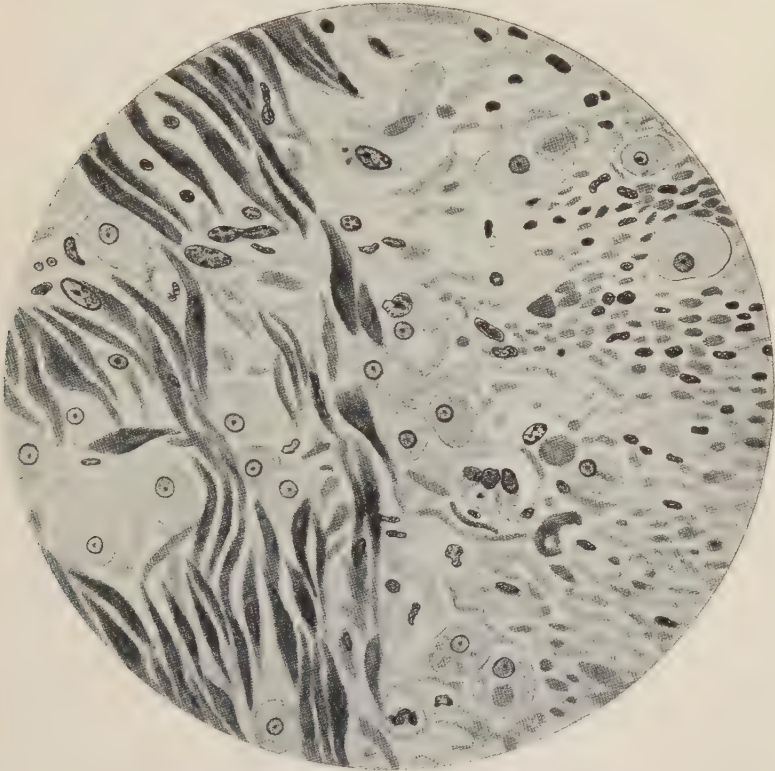


FIG. 7.—*Endamœba histolytica*. Section of wall of colon in the margin of an amebic ulcer. The amebæ (*E. histolytica*) are observed in the connective tissue of the submucosa and in the muscular layer of the intestinal wall.  $\times 560$ . (After Kofoid and Swezy.)

shreds of necrotic tissue, blood corpuscles and leucocytes, degenerated liver cells, and amebæ. The abscess wall is covered internally with shreds of necrotic tissue, and, if the abscess is small, shreds of connective tissue reach across the abscess cavity. I have observed cases in which all trace of liver substance within the abscess had been lost, except the connective tissue framework of the organ, which, being more resistant to the cytolytic action of the amebæ than the other elements of the liver tissue, still persisted as shreds of tissue crossing the abscess cavity. The amebæ are found in small numbers in the contents of the abscess, but occur in large numbers in that portion of the abscess wall

nearest the cavity. Secondary infections with bacteria are frequent, and then the abscess loses its characteristic appearance and resembles the abscesses caused by the pyogenic cocci to a much greater extent.

Abscess of the liver may occur in individuals who have never suffered from an attack of dysentery, but in all such cases the primary infection is in the intestine.

*The Spleen.* Amœbic abscess of the spleen has been reported by Maxwell (1909) and Rogers (1913), but is a very rare condition.

*The Lung.* Amœbic abscesses of the lung have been reported by several observers and generally follow the rupture of a liver abscess in that organ. I have observed five cases in which rupture of a liver abscess occurred into the pleura. In amœbic abscess of the lung the amœbæ occurred in the sputum, which is blood-stained, and the amœbæ may contain red blood corpuscles. Primary amœbic abscess of the lung is a very rare condition, but may occur as a complication of amœbic infection of the intestine.

*The Brain.* Amœbic abscess of the brain is a very rare condition, but such cases have been reported by Legrand (1912), Armitage (1919), and others.

*The Bladder.* Invasion of the walls of the bladder by *Endamæba histolytica* rarely occurs, but several authentic cases are on record. I observed one instance of such infection in which the infection of the bladder was caused by a minute fistula connecting the bladder with an amœbic ulceration of the intestine, which was adherent over the bladder. This condition was discovered at autopsy, but the urine of this patient had shown motile amœbæ, some of which contained red blood corpuscles, for some days before the patient died of amœbic dysentery. The bladder wall in the neighborhood of the fistula was necrotic, and amœbæ were demonstrated in the tissues.

*The Urine.* Several authorities have reported the presence of *Endamæba histolytica* in the urine. Some of these reports are undoubtedly erroneous, but there is no doubt that this parasite has been found in the urine following infection of the urinary tract, but in all such cases the primary infection was in the intestine. Authentic instances have been reported by Bælz (1883), Craig (1911), Fischer (1914), Walton (1915), and Macfie (1916).

*The Testis and Epididymis.* Warthin (1922) has reported a case in which *Endamæba histolytica* occurred in the testis and epididymis of a patient who had suffered from amœbic dysentery, and who died from pneumonia after an exploratory operation for suspected liver abscess. The parasites were found in the tissues of the testis and epididymis, but were especially numerous in the ducts. In the epididymis the amœbæ were very numerous in the ducts and many of them contained red blood

corpuscles. In the testis the amœbæ were found especially in the seminiferous ducts, in which situation the amœbæ had phagocyted the spermatozoa. Superficial ulceration was noted in the ducts of the epididymis in places, and pre-cystic and cystic forms of the amœbæ, as well as adult forms, filled with red blood corpuscles, were present in such localities. This observation is the first on record of invasion of the testis and epididymis by *Endamœba histolytica*.

*The Glands.* Kofoid, Boyers, and Swezy (1922) record the presence of *Endamœba histolytica* in the glands in Hodgkin's disease, but their observations await confirmation.

*The Bones.* Kofoid and Swezy (1922) and Ely, Reed, and Wyck-off (1922) have recorded the finding of *Endamœba histolytica* in the bone lesions of non-bacterial arthritis deformans. The diagnosis of the parasite was based upon the type of mitosis and the number of chromosomes in the cells, and the authors believe that the cells observed by them were identical with *Endamœba histolytica*. Their results await confirmation.<sup>2</sup>

**Prophylaxis of *Endamœba histolytica*.**—The prophylaxis of infection with *Endamœba histolytica* depends upon the proper care and disposal of human excreta and the observance of the well-known rules relating to personal hygiene. It has been shown that the cysts, which are the infective agents, are voided in the fæces and can live therein, under favorable conditions, for several weeks. The immense number of cysts that may be voided by a single "carrier" in a day may be understood by a consideration of the results of Kofoid and his co-workers. They observed one "carrier" for 42 days, the stools being examined upon 26 days. The number of cysts in a given portion of stool, diluted with salt solution, was counted in a blood-counting chamber, and the number then calculated for the whole stool. They found that on 26 of the 42 days the number varied from 333,000 to 45,000,000 per day, the average being 14,520,000 per day for the 26 days, and 8,145,000 for the 42 days. These figures, while, of course, only approximate, indicate the menace of the "carrier" so far as the number of cysts which he may excrete is concerned, and the importance of rendering his fæces harmless.

The best method of disposing of the fæces of "carriers" is through properly constructed sewers, but where this is impossible the fæces should be disinfected before being disposed of. This is best accomplished by mixing the stool with a solution of cresol containing one part of cresol to 200 parts of water and allowing the mixture to stand for 15 minutes. As already noted, cresol is the only chemical that has been found practically efficient in killing the cysts of this parasite, and it should al-

<sup>2</sup> Smith, S. (1924, *Jour. Roy. Army Med. Corps.* XLII, 438), reports a case of arthritis of the left knee-joint in which *E. histolytica* was demonstrated in the pus from the joint. The amœbæ were actively motile.



ways be used for the disinfection of the stools, when such disinfection is necessary.

Owing to their number, it is impossible to isolate "carriers" of *Endamæba histolytica*. The vast majority of patients convalescent from amœbic dysentery become "carriers" despite the most energetic treatment with emetine. In the epidemic at El Paso, already referred to, of 115 patients suffering from amœbic dysentery, no less than 86, or 74.7 per cent., developed cysts and became "carriers" of the infection, despite the most energetic treatment with emetine, in some cases two courses of 12 grains of the drug having been administered. In view of this fact and that a much larger number of individuals who have never had symptoms of dysentery are "carriers" of the infection, the proper disposal of human fæces, or its disinfection, if it cannot be disposed of without danger of contaminating food and drink, is the most essential step in the prophylaxis of this parasite. The disinfection of the fæces is of special importance where it is possible for flies to gain access to the excreta.

The prevention of the contamination of food by the soiled hands of "carriers" is very important, and to insure this no "carrier" of *Endamæba histolytica* should be employed as a food handler. In the United States Army the fæces of all food handlers are carefully examined for cysts of this parasite, and if these are found such individuals are recorded and are never allowed to work in mess halls or kitchens of organizations.

The prevention of the breeding of flies and the destruction of these insects when present is a prophylactic measure of great value. The use of fly-traps, fly-paper, and the fly-"swatter" should be encouraged wherever amœbic dysentery is endemic, if flies are present in any number.

The screening of houses, kitchens, mess halls, and of exposed food to prevent access of flies is of the greatest importance in prophylaxis, where it is impossible to prevent the breeding of these insects.

In regions where amœbic dysentery is present, especially in the tropics, where the use of human excrement as a fertilizer is often employed, the eating of uncooked fruits, vegetables, and salads in which either uncooked fruit or vegetables are used, should be strictly prohibited. Lettuce is especially dangerous under the conditions mentioned and should never be used where amœbic dysentery is present to any marked extent.

Individuals who are "carriers" of this parasite should be informed of the danger of their transmitting it to others and of the reinfection of themselves, and should be instructed regarding the measures that they may take to prevent such transmission or reinfection.

Amœbic dysentery is a strictly preventable disease, and the well-



recognized sanitary measures now taken in the prevention of the typhoid fevers, bacillary dysentery, and cholera are equally efficient in the prevention of infection with *Endamæba histolytica*. Present knowledge regarding this infection demonstrates that in its epidemiological features it is strictly comparable with the diseases mentioned and that the same general methods of prophylaxis are indicated, and, if properly enforced, will meet with the same measure of success. There is nothing mysterious about the origin and transmission of this disease, but if success is to be attained in its prophylaxis the important part played by "carriers" and flies in its transmission must be constantly remembered.

It should not be forgotten that a water supply which is contaminated cannot be rendered safe for domestic use by chlorination, as the cysts of *Endamæba histolytica* are not affected by chlorine in the amounts employed in water sterilization, and that the only safe method of rendering such water safe is by boiling.

**Diagnosis.**—The diagnosis of *Endamæba histolytica* is considered in Chapter V.

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NOTE.—The bibliography of the parasitic amœbæ is now enormous and only the papers quoted from in the test are here included. For a complete bibliography up to, and including, 1919, see Dobell's "The Amœbæ Living in Man."

## CHAPTER III

THE PARASITIC AMŒBÆ OF MAN (CONTINUED). *ENDAMŒBA COLI*.  
*ENDAMŒBA NANA*. *ENDAMŒBA GINGIVALIS*.

IN THIS chapter will be described the three remaining species of the genus *Endamæba*, *Endamæba coli*, *Endamæba nana*, and *Endamæba gingivalis*.

Species II. *ENDAMŒBA COLI* (Grassi, 1879), Hickson, 1909.

The synonyms of *Endamæba coli* are very numerous but the principal are the following:

Synonyms: "Amœba coli," Lösch, 1875. *Amœba coli*, Grassi, 1879. *Entamæba coli*, Casagrandi and Barbagallo, 1897. *Entamæba hominis*, Casagrandi and Barbagallo, 1897. *Entamæba coli*, Schaudinn, 1903. *Entamæba hartmanni*, Prowazek, 1912, *pro parte*. *Entamæba brasiliensis*, Aragão, 1912, *pro parte*. *Entamæba coli communis*, Knowles and Cole, *pro parte*. *Endamæba intestino vulgaris*, Aragão, 1917. *Endamæba coli*, Craig, 1917. *Endamæba hominis*, Pestana, 1917.

**History and Nomenclature.**—According to Dobell (1919) *Endamæba coli* was probably discovered by Lewis (1870) in the stools of patients suffering from cholera in India, but his descriptions were not accurate enough to make the identification of the parasite certain. His co-worker, Cunningham, however, in the same year published observations which Dobell states furnished conclusive evidence that he actually saw and described *Endamæba coli*. However this may be, we know that Grassi, in 1879, saw and described this parasite in the stools of both healthy individuals and those suffering from diarrhoeal and other diseases, although he erroneously identified this amœba with the amœba described by Lösch, and following him, named it *Amœba coli*, Lösch.

There is no question that Grassi was working with *Endamæba coli*, although there is evidence that some of the material he examined also contained *Endamæba histolytica*, and that the greatest part of his description refers to this species, which, of course, is not identical with the amœba that Lösch described and which we now know to have been *Endamæba histolytica*.

Quincke and Roos (1893) and Roos (1894) confirmed and extended Grassi's observations, and accurately described the motile forms, or trophozoites, distinguishing them from similar forms of *Endamæba histolytica*. They also observed the cysts of *Endamæba coli* but did not determine the number of nuclei in these cysts.

Casagrandi and Barbagallo (1895-97) confirmed the work of Grassi and Quincke and Roos upon this amœba, and first named it *Entamæba*



*coli*, and later, *Entamæba hominis*, thus establishing a new generic name, apparently in ignorance of the generic name *Endamæba*, established in 1879, by Leidy. These investigators observed and described both the motile and encysted forms of *coli* but did not ascertain the number of nuclei in the cysts, and in parts of their descriptions confused *Endamæba coli* with both *Endamæba histolytica* and *Endamæba nana*, as some of their material contained both of these organisms.

In 1903, Schaudinn published the results of his researches upon the parasitic amœbæ of the human intestine and redescribed this parasite. Schaudinn's description was in many respects erroneous and led to great confusion, but to him we owe the first accurate description of the number of nuclei in the cysts, which he determined to be eight in the fully developed cysts. The importance of this fact in the differential diagnosis of the species from *Endamæba histolytica*, in which the cysts contain four nuclei, was first insisted upon by Schaudinn, and although all of the authorities mentioned prior to Schaudinn considered *Endamæba coli* as a non-pathogenic amœba, the acceptance of this fact by Schaudinn greatly strengthened the position of those who claimed that both pathogenic and non-pathogenic amœbæ occurred in the intestine of man and led to greatly increased research upon these parasites.

After Schaudinn's publication many observers contributed studies of *Endamæba coli* which were, in the main, confirmatory of his results, but it was proven that his description of reproduction, both of the motile and encysted forms, were incorrect.

In 1913, Walker and Sellards definitely proved that *Endamæba coli* is a harmless commensal in the human intestine by actual experiments upon man by feeding volunteers with material containing the cysts of this species.

The nomenclature of *Endamæba coli* is in the same confused state as that of *Endamæba histolytica*, a condition caused by the acceptance, by Schaudinn, of the specific name "*coli*" for the harmless amœbæ when he should have applied that name to the pathogenic amœba, which he named *Endamæba histolytica*, for there is no doubt that the amœba described by Lösch and called "*Amæba coli*" is identical with the pathogenic amœba named by Schaudinn "*Endamæba histolytica*." Had Schaudinn accepted the specific name "*coli*" for this amœba all confusion would have been avoided, but the name *coli*, as indicating the non-pathogenic parasite, has now become so firmly fixed in the literature that a change is inadmissible, as it would only result in still greater confusion and accomplish no good purpose beyond fulfilling strictly the laws of nomenclature, which, in such a case, should be disregarded.

As indicated in the list of synonyms, *Endamæba coli* has been rediscovered by several observers and renamed under the belief that they

were observing new species of amœbæ. The following supposed new species are identical in whole or in part with *Entamœba coli*: *Entamœba hartmanni*, Prowazek, 1912. *Entamœba brasiliensis*, Aragão, 1912. *Entamœba nipponica*, Koidzumi, 1909. *Entamœba coli communis*, Knowles and Cole, 1917.

**Morphology.**—*Entamœba coli*, like *Entamœba histolytica*, has a vegetative, pre-cystic, and cystic stage in its life-cycle. The vegetative,



FIG. 8.—*Entamœba coli*.  $\times 2,000$ . (After Dobell and Boeck and Stiles.) Large, motile, vegetative form, containing numerous ingested bacteria (bact.) nucleus with karyosome (kary.), nuclear meshwork or linin net-work (m.w.), peripheral chromatin (p. chr.), and vacuoles (vac.).

or motile stage, occurs only in liquid and semi-liquid stools, the pre-cystic stage in semi-formed or formed stools, and the cystic stage in formed stools, as a general rule.

#### 1. Vegetative Stage.—

##### a. Living Specimens. In

the living condition this amœba ap-

pears as a sluggishly moving amœba containing a visible nucleus, numerous vacuoles, and much ingested material within its cytoplasm.

The size varies somewhat as given by different authorities. Schaudinn (1903) gave the diameter as between 8 and 50 microns; Dobell (1919) states that the diameter of the resting vegetative forms varies from 18 to 40 microns, specimens as a rule measuring between 20 and 30 microns. Boeck (1923) gives the diameter of the resting trophozoites as between 20 and 40 microns, the average being between 20 and 30 microns; Hegner and Taliaferro (1924) give the diameter as varying between 18 and 40 microns, the average as being between 20 and 30 microns. It is very evident that this parasite must have very uniform measurements or else authorities have copied the averages given by others. In my experience the size of this species varies more than is indicated by the above measurements, as I have observed undoubted individuals of this species measuring as little as 15 microns in diameter

and as much as 50 microns in diameter, when in the vegetative stage of development and motionless. I have found the size to vary from 15 to 50 microns in diameter, the average organisms measuring between 20 and 30 microns. In size this species closely resembles *Endamæba histolytica*, the latter being slightly larger, as a rule, but size is of no value in the differentiation of the two species.

The *cytoplasm* of *Endamæba coli* appears of a grayish or slightly greenish hue, and there is very little distinction between the ectoplasm and endoplasm when the organism is moving, and none at all when it is motionless. The ectoplasm of this species never presents the clear, hyaline, glass-like appearance so characteristic of the ectoplasm of *Endamæba histolytica*, the pseudopodia in this species being with difficulty distinguished from the remainder of the organism except by the motion. The lack of distinction between the ectoplasm and the endoplasm in *Endamæba coli* is one of the most important points in the differential diagnosis between this amœba and *Endamæba histolytica*.

The *endoplasm* of this species is granular in appearance and contains a visible nucleus, many vacuoles, and much ingested material.

The *nucleus* is almost always visible as a ring of coarse granules, highly refractile, enclosing a refractile rounded mass of chromatin, the karyosome, which is situated to one side of the centre of the nucleus, instead of centrally, as in *Endamæba histolytica*. In degenerating amœbæ refractile grains of chromatin may be distributed throughout the nucleus or collected in irregular masses within it, but such appearances are not observed in healthy organisms. In some specimens a few grains of refractile chromatin may be seen between the karyosome and the ring of granules representing the nuclear membrane.

*Vacuoles* are invariably present in the cytoplasm of this species, unlike *Endamæba histolytica*, in which vacuoles are present only in degenerating specimens. Some of these vacuoles contain ingested food materials, while others are apparently empty. Certain vacuoles occur in this species that are characteristic, resembling rents or tears in the cytoplasm and pointed at the ends, but their nature is uncertain. Some believe that this type of vacuole is produced by the degeneration of the parasite, but they apparently occur in perfectly healthy organisms, so far as I have been able to observe.

*Ingested material* is always present in the cytoplasm of *Endamæba coli* during the vegetative stage of existence and varies greatly in character. This amœba feeds actively upon nearly all substances that are present in the lumen of the intestine except tissue cells and red blood corpuscles. The cytoplasm and food vacuoles are generally filled with bacteria, crystals, vegetable cells, or other food material. Both unencysted and encysted protozoa may also be present, as *Trichomonas*

*hominis*, *Giardia intestinalis*, *Chilomastix mesnili*, and even cysts and pre-cystic forms of *Endamæba histolytica*. The ingested material may lie within food vacuoles or apparently free in the endoplasm.

The fact that this species does not ingest red blood corpuscles is of great assistance in the differentiation of it from other amœbæ of the intestine of man and especially from *Endamæba histolytica*, the species with which it is most often confused. Several investigators, including myself, have reported the presence of red blood corpuscles in *Endamæba coli*, but I am now convinced that I mistook specimens of *Endamæba histolytica* containing these cells for *Endamæba coli*, a mistake readily made if one was observing degenerating forms of the former species, which happened to contain red blood corpuscles. I think that the same mistake has been made by the other observers who have reported red blood cells within *Endamæba coli*, as mixed infections with the two amœbæ are very common, and such a mistake could easily be made. Experimentally, it is impossible to make *Endamæba coli* ingest red blood corpuscles by adding blood to material containing it, and it is very doubtful if this species is phagocytic for these cells under any condition. At any rate, if *Endamæba coli* does ingest these cells it is so very rarely as to be of no practical importance as far as the differential diagnosis of the two species is concerned. I am not prepared to state that the ingestion of red blood corpuscles by this species of amœba never occurs under favorable conditions, for Yorke and Macfie (1919) have demonstrated that even a free-living amœba that they cultivated for four years ingested red blood corpuscles when grown on a medium containing fresh blood, and it may well be that in certain cases in which blood is free in the lumen of the intestine, *Endamæba coli* may rarely ingest the red blood corpuscles. However, it is a safe diagnostic rule to regard any amœba occurring in the fæces of man and containing red blood corpuscles as *Endamæba histolytica*.

The endoplasm of *Endamæba coli* contains many bacteria, as these apparently form the bulk of the food of this parasite. This distinguishes this species from *Endamæba histolytica*, in which the endoplasm never contains bacteria unless degeneration is occurring.

A contractile vacuole never occurs in this species. *Motility.* *Endamæba coli* is a sluggishly motile amœba in which progressive motion is often absent, and when present, is of short duration and very limited in extent. Progressive motility is only present in amœbæ in freshly voided fæces. The pseudopodia are formed by the ectoplasm and are small and blunt, and there is no well-marked boundary between the ectoplasmic pseudopodia and the endoplasm, as in *Endamæba histolytica*. This amœba never presents the very refractile, long, finger-like pseudopodia which are so characteristic of the latter species, and the pseudo-



podia are not extruded rapidly and explosively, as in *Endamæba histolytica*, but slowly and uncertainly, often being withdrawn before the endoplasm flows into them.

Two forms of motility are observed: the first consisting of the slow protrusion of pseudopodia into which flows the endoplasm, thus producing a sluggish progressive motion; the second consisting of the protrusion of pseudopodia which are withdrawn before the endoplasm flows into them, thus causing a change in the shape of the amœba, but with no progressive motion.

The sluggish motility of *Endamæba coli* is in marked contrast to the very active motility, progressive in character, of *Endamæba histolytica*, and is a very valuable aid in the differentiation of the two species. However, it is only in freshly voided fæces that motility is pronounced in either species, although that of *Endamæba histolytica* is retained for a longer time and may be present in fæces that have been kept at room temperature for an hour or more in warm weather. Under such conditions motility is not usually present in *Endamæba coli*.

*b. Stained Specimens.* The staining reactions of *Endamæba coli* with the iron-hæmatoxylin methods are like those of *Endamæba histolytica*, the cytoplasm staining a brownish or grayish-brown color, while the nuclear membrane, karyosome, and chromatin granules in the nucleus stain black. In stained specimens of the vegetative stage, or trophozoite, of this species, the character of the nucleus is of special importance in diagnosis.

The nucleus, in stained specimens, is observed to be much richer in chromatin than that of *Endamæba histolytica*, but the typical structure of the nucleus is only observed in specimens stained within an hour after the fæces have been voided, as after this period degeneration occurs to such an extent that the typical structure is lost.

The nucleus is round in shape, or very slightly oval, in rare instances, and measures from four to eight microns in diameter, being larger than the nucleus of *Endamæba histolytica*, as a general rule. The nuclear membrane is thicker than that of the latter species and is covered internally by a single layer of chromatin granules that are also larger than those lining the nuclear membrane of *Endamæba histolytica*. In amœbæ in which degeneration is occurring, the chromatin lining the nuclear membrane collects into large, irregular masses upon the inner surface of the membrane and often appears considerably increased in amount.

The karyosome appears as a solid black, round mass situated to one side of the centre of the nucleus. The eccentric situation of the karyosome in this species serves to distinguish it from *Endamæba histolytica*, in which the karyosome is central in position. The karyosome in this species may appear central, in some of the amœbæ, due to the

angle at which the organism may be viewed, but the typical situation is eccentric.

Surrounding the karyosome is an unstained area, or halo, similar to that present in *Endamæba histolytica*, but larger and more clearly differentiated. In the space between the unstained area and the nuclear mem-

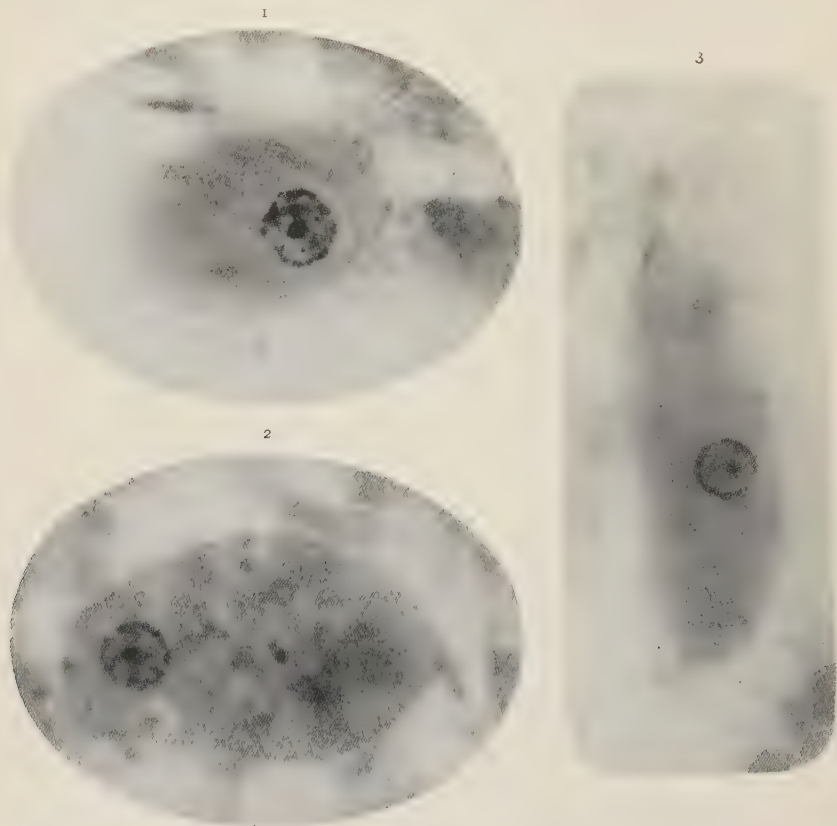


FIG. 9.—*Endamæba coli*.  $\times 1,200$ . (Photomicrographs, from Army Medical School Collection.) Stained with iron-hæmatoxylin. 1. Vegetative form of *Endamæba coli*. Note large karyosome and clumps of chromatin on inner side of nuclear membrane. 2. Vegetative form, or trophozoite, of *Endamæba coli*. Note thick nuclear membrane and eccentric situation of karyosome. 3. Vegetative form, or trophozoite, of *Endamæba coli*. Very large specimen showing typical nucleus. Note large karyosome, granules of chromatin on inner side of nuclear membrane, and chromatin granules between membrane and karyosome.

brane a few deep-black grains of chromatin are almost invariably present and traces of a linin net-work may be observed. The chromatin between the karyosome and the nuclear membrane in this species also serves to distinguish it from *Endamæba histolytica*, in which chromatin granules never occur in this situation unless the organism is degenerating.

In degenerating specimens of *Endamæba coli* the chromatin between the karyosome and the nuclear membrane is increased in amount and the karyosome often appears split into several minute grains of chro-

matin. Similar appearances are met with in the nucleus of degenerating specimens of *Endamæba histolytica* and it is impossible to differentiate the two species after degeneration is at all advanced.

The ingested material in stained specimens is well differentiated and, as already stated, the large number of bacteria, and the presence of yeast and other vegetable cells, serve to distinguish this species from *Endamæba histolytica*, in which the cytoplasm is free from such inclusions. The unencysted and encysted protozoa sometimes contained within *Endamæba coli* are well shown in stained preparations.

2. **Pre-cystic Stage.**—*a. Living Specimens.* Prior to encystment, *Endamæba coli* becomes reduced in size, rids itself of all ingested material, and produces the forms known as pre-cystic forms. In the fæces these forms appear as hyaline bodies, which are immotile or very sluggishly motile. Usually if any motion is present it consists largely of the slow extrusion of small, blunt pseudopodia and their withdrawal, without any progressive motion resulting. The nucleus, in these forms, is very distinct in the living specimen, consisting of a ring of refractile granules containing a round, refractile mass, situated eccentrically, the karyosome. The pre-cystic forms measure from 15 to 22 microns in diameter, the size varying with the size of the cyst which they will produce. At this stage of development it is generally impossible to differentiate the pre-cystic forms of this species from those of *Endamæba histolytica*, as the nuclear characters of both species at this time are very similar. Fortunately, cysts are usually present if pre-cystic forms occur in the fæces, and one can make the diagnosis from the study of the cysts.

*b. Stained Specimens.* The staining reactions of the pre-cystic forms are like those of the vegetative forms already described.

The structure of the nucleus is similar to that of the trophozoite, there being the same eccentrically situated karyosome and the occurrence of chromatin granules between the karyosome and the nuclear membrane. However, at this stage of development the nucleus often resembles that of the pre-cystic forms of *Endamæba histolytica*, and a differential diagnosis is frequently impossible. The eccentric situation of the karyosome, when present, is diagnostic of the pre-cystic forms of *Endamæba coli*, but specimens are often observed in which the karyosome is almost or quite central in position, as in *Endamæba histolytica*.

3. **Cystic Stage.**—*a. Living Specimens.* The pre-cystic forms of *Endamæba coli* eventually secrete a cyst wall, which surrounds the organism and appears as a very refractile hyaline membrane having a double outline and considerably thicker than the cyst wall of *Endamæba histolytica*. In the living condition the cysts appear as refractive colorless bodies, round or slightly oval in shape. Rarely cysts are observed which are irregular in shape, but such cysts I believe to be undergoing

degeneration, or the shape altered during the preparation of the specimens by pressure.

The *size* of the cysts of this species varies greatly, and there is good evidence that there exist definite races that produce cysts of different size, as in *Endamæba histolytica*. Dobell (1919) states that the cysts vary in size from 10 to 30 microns, or more, in diameter, and that he has never seen a cyst of this species smaller than 10 microns in diameter or larger than 33.5 microns in diameter, although larger cysts have been observed by others. Boeck (1923) gives the size of the cysts as between 12 and 22 microns in diameter, the average cysts measuring between 15 and 18 microns in diameter. Hegner and Taliaferro (1924) give the size of the cysts as between 10 and 30 microns in diameter or larger. In my own experience the size of the cysts of *Endamæba coli* varies from 10 to 30 microns in diameter, but I have rarely observed cysts measuring as much as 30 microns, the usual limit of size being about 22 microns in diameter, and the vast majority of the cysts of this species that I have observed measured from 12 to 18 microns in diameter.

Mathews (1919) has demonstrated that there are probably four races of *Endamæba coli* producing cysts of distinctive size. These races produce cysts having a mean measurement of  $15\mu$ ,  $16.5\mu$ ,  $18.7\mu$ , and  $21.7\mu$ , respectively. Dobell (1919) agrees with Mathews' conclusions regarding the existence of such races, and Boeck (1923) states that there are probably three size races having cysts from  $12\mu$  to  $14\mu$ ,  $15\mu$  to  $18\mu$ , and  $19\mu$  to  $22\mu$  in diameter.

While the cysts of *Endamæba coli* are usually considerably larger than those of *Endamæba histolytica*, the size of the cysts of the two species is of little value in distinguishing them. However, as pointed out by Dobell (1919), a cyst measuring less than 10 microns in diameter is probably not a cyst of *Endamæba coli*, while one measuring more than 20 microns in diameter is probably not a cyst of *Endamæba histolytica*.

The cytoplasm of the cysts is free from ingested material, but the nuclei may often be distinguished in the living specimen as refractile rings composed of dots of chromatin, while the karyosome appears as a refractile round mass, eccentric in position. Chromidial bodies are sometimes visible as needle-like or filamentous, or rod-like, very refractile bodies, often arranged in a sheaf-like manner in the cytoplasm. In cysts stained with the iodine solution, the nuclear membrane, the karyosome, and the chromidial bodies are more plainly seen, and the number of nuclei can be easily counted. (For illustration of cysts see Chapter V.

In some of the cysts a very large, somewhat irregular area may be seen, less refractive than the surrounding cytoplasm, which, when



stained with the iodine solution, takes a dark-brown or port-wine color, and which is known as the *glycogen mass*. The amount of this substance in the cysts of *Endamæba coli* is much greater than in the cysts of *Endamæba histolytica*, and it is most abundant in the young cysts, especially in those having two nuclei. As the development of the cyst progresses, the glycogen, which apparently serves as reserve food material, gradually disappears, and the fully developed 8-nucleate cyst contains little or none of this substance. Mathis and Mercier (1917) and Wenyon and O'Connor (1917) regard the cysts containing glycogen as incapable of further development, but Dobell (1919) does not agree with this opinion and points out the fact that as the vast majority of the cysts of this species contain glycogen, it is impossible to believe that all of these cysts are sterile so far as further development is concerned. It is undoubtedly true that the opinion of Dobell is correct and that the presence of glycogen within the cysts of *Endamæba coli* is a normal phenomenon in the development of this stage in the life-cycle of the amœbæ. Glycogen may also be demonstrated in some of the pre-cystic forms which later develop into cysts.

*b. Stained Specimens.* The structure of the cysts of *Endamæba coli* is best studied in specimens stained by one of the hæmatoxylin methods. When so stained, the cyst wall remains unstained, the cytoplasm stains a grayish-brown or bluish color, while the nucleus and chromidial bodies stain black. The glycogen mass does not stain but appears as a large vacuole in the cytoplasm.

The *nuclei*, in stained specimens, resemble in general structure the nucleus in the pre-cystic and vegetative forms, but vary in size with the number of nuclei in the cyst. They are circular in shape, unless dividing, when they are spindle-shaped, and such nuclei are sometimes seen in a cyst along with round nuclei. The thicker nuclear membrane, the eccentric situation of the karyosome, and the presence of granules of chromatin between the karyosome and the nuclear membrane are all repeated in miniature in even the cysts of *Endamæba coli* having 8 nuclei, thus rendering the differentiation of the cysts of this species from those of *Endamæba histolytica* possible, even if the cysts of *coli* show only 4 nuclei.

Most of the cysts of *Endamæba coli* contain 1, 2, or 8 nuclei, cysts containing 4 nuclei being comparatively rare. Cysts are also observed containing 3, 5, and 7 nuclei, due to unequal division, and cysts containing more than 8 nuclei are also observed. I have seen cysts of this species containing as many as 16 nuclei, and Dobell (1919) states that he has observed cysts containing 18 and 20 nuclei. However, the vast majority of the cysts of this species contain 8 nuclei, and cysts with more than this number are rare.

The nucleus in the uninucleate cyst is generally comparatively large and very rich in chromatin, as shown by the thick nuclear membrane, the large granules lying between the membrane and the karyosome, and the size of the granules lining the nuclear membrane. In the binucleate amœbæ the nuclei are smaller, although similar in structure, and a progressive diminution in the size of the nuclei occurs as division progresses. In the 8-nucleate amœbæ the diameter of the nuclei is not more than  $1/4$  to  $1/6$  of the diameter of the cyst, while in the uninucleate amœba the diameter of the nucleus may be over  $1/3$  that of the entire cyst. In the cysts containing 3, 5, or 7 nuclei, one of the nuclei is generally about twice as large as the other nuclei present, due to the fact that this nucleus has not divided. This is especially well marked in the trinucleate cysts, in which one nucleus is generally at least twice the size of the other two nuclei present.

*Chromidial bodies* are present in the cytoplasm of about 10 per cent. of the cysts of *Endamæba coli*, in my experience, although Smith (1918) states that only 5.5 per cent. of the cysts of this species show these bodies. They stain black, and are entirely different in appearance from the chromidial bodies seen in the cysts of *Endamæba histolytica*. They occur as filamentous, acicular, or staff-like bodies, having square or pointed ends, and frequently form sheaf-like masses which resemble a sheaf of acicular crystals. Rarely the cyst may contain long filamentous threads which almost fill it, the nuclei lying between the filaments. Smaller granular or rod-like chromidial bodies may also be present which might be mistaken for bacteria by one unused to observing these cysts.

Chromidial bodies are most numerous in the young cysts and I have rarely observed them in the pre-cystic forms in the shape of granular or rod-like masses. The binucleate cysts appear to be richer in chromidial bodies than other cysts and they are practically absent from the 8-nucleate cyst. In some of the latter the small rod-like chromidial bodies may sometimes be observed. I have never observed chromidial bodies in the supernucleate cysts.

The chromidial bodies in the cysts of *Endamæba coli* are easily differentiated from those that occur in the cysts of *Endamæba histolytica*, which are large oval, or rod-like masses of chromatin with rounded ends, and the character of these bodies in the cysts is a most important and valuable differential point in the diagnosis of the two species of amœbæ.

In specimens stained with hæmatoxylin the glycogen mass remains unstained and appears as a large vacuole in the cytoplasm. This vacuole occurs generally in the young cysts up to, and including, the binu-

cleate stage of division, and I have never observed it in the eight-nucleate cyst.

**Resistance of the Cysts.**—The cysts of *Endamæba coli* are even more resistant to physical agents than are those of *Endamæba histolytica*, as shown by the experimental data available.

*Survival in the Fæces.* Walker and Sellards (1913) found that the cysts of this species were infective for man after having remained in the fæces for ten days, and Thompson and Thompson (1916) found that the cysts remained unchanged in the fæces for one month, as did Wenyon and O'Connor (1917).

*Survival in Water.* If fæces containing the cysts of this species be greatly diluted with distilled water the cysts have been found viable after a period of five weeks. If the cysts are removed from the fæces by filtration and washing, and the washed cysts then placed in distilled water and kept at a temperature between 12° and 22° C. (53.6° and 71.6° F.), it was found by Boeck (1921) that some of the cysts were viable after 244 days.

*Thermal Death Point of Cysts.* The thermal death point of the cysts of *Endamæba coli* has been determined by Boeck (1921) to be 76° C. (168.8° F.), the highest thermal death point of any of the cysts of the intestinal protozoa.

*Resistance in Intestine of Flies.* Wenyon and O'Connor (1917) found that the cysts of this species of amœba survived in the intestine of the fly for forty-two hours, and that the intestine of flies caught wild contained the cysts of this parasite. Living cysts were demonstrated in the fæces of flies for sixteen hours after feeding upon infected material.

Roubaud (1918) found that cysts of this amœba were excreted in the fæces of flies, in a viable condition, for over twenty-four hours after feeding and rarely for over forty-eight hours.

Root (1921), in a careful and accurate series of observations, ascertained that the cysts of *Endamæba coli* in the intestine of 75 flies he experimented with, began to die at the end of two hours after feeding; that half of the cysts were dead in fourteen, sixteen, and eighteen hours, in the three different series of experiments, and that the last living cyst was found in the intestine of a fly fifty-two hours after feeding.

**Number of Cysts of *Endamæba coli* in Stools.**—The number of cysts of *Endamæba coli* that may be excreted in the fæces of an infected individual in a day may be enormous. Cropper (1919) counted the cysts of this species occurring in the stools by a special and accurate method and found that the smallest number was 3,250 cysts per granime of fæces, and the highest count was 323,690 cysts per gramme. The

number of cysts excreted in a single day varies from 290,000 to sixty-four million.

**Habitat.**—The normal habitat of *Endamæba coli* is in the large intestine of man and it never invades any other part of the human body. The vegetative forms, or trophozoites, are found in the upper part of the colon, where the fæces are fluid, the pre-cystic forms are found lower down the intestine, where the fæces are semi-formed, and the cysts are found in the lower portion of the intestine, where the fæces are formed. Whether this species occurs as a parasite in any of the lower animals is still uncertain, as monkeys are said to be infected with an amœba indistinguishable morphologically from this species, but definite proof is still lacking that it is identical with *Endamæba coli*.

**Species Occurring in Lower Animals.**—As stated, a species of amœba indistinguishable from *Endamæba coli*, in morphology, has been observed in monkeys by Brumpt (1904), Wenyon (1908), Pro-wazek (1912), Mathis (1913), Behrend (1914), and Mathis and Mercier (1917). This amœba was named *Entamæba pitheci* by Pro-wazek, in 1912, and is identical with *Löschia legeri*, Mathis, 1913, and *Entamæba legeri*, Mathis and Mercier, 1917. Dobell (1919), who has studied the cysts of this amœba in the fæces of a monkey, states that he is unable to distinguish them from those of *Endamæba coli*.

**Cultivation.**—Although several observers have claimed success in the cultivation of this species it is now known that the amœbæ which they cultivated were really free-living amœbæ which had passed through the intestinal canal of man in the cystic form or had reached the fæces after passage, as such contamination is very common, especially in tropical and subtropical regions. At the present time there is no evidence that *Endamæba coli* has ever been cultivated *in vitro*.

**Life-history.**—The life-history of this species of amœba includes a vegetative, or motile, stage, a pre-cystic stage, and a cystic stage. The cysts, upon being swallowed by man, pass unchanged through the stomach, and either in the small intestine or in the upper limits of the large intestine liberate eight small amœbæ or an 8-nucleate amœba which afterwards divides. Whether excystation occurs in the small or large intestine, or whether eight small amœbæ are liberated from the cyst, or an 8-nucleate amœba, are points in the life-history that still remain undetermined.

The amœbæ liberated from the cysts are called trophozoites, and live and multiply in the fluid contents of the upper portion of the large intestine. These forms are constantly being carried down the intestine and when they reach a portion where the fæces are semi-formed, motility is lost and pre-cystic forms are produced. Whether these pre-cystic



forms are derived directly from the large motile forms or whether they are produced by the multiplication of similar forms is still undetermined.

When the pre-cystic forms reach the lower portion of the large intestine, where the fæces are formed, they secrete a cyst wall and become cysts, which are the infective agents. The cysts are excreted in the stools of the infected individual and undergo no further development unless swallowed by man, when the life-history outlined above is repeated.

**Method of Reproduction.**—In the vegetative stage *Endamœba coli* reproduces by simple fission, the division of the nucleus into two being followed by the binary division of the body of the amœba. In the cystic stage multiple fission of the nucleus occurs, the nucleus dividing into two, these two into two, producing a 4-nucleate cyst, and finally these four into two each, producing the 8-nucleate cyst characteristic of the species.

Dividing vegetative forms have been described by Schaudinn (1903), Craig (1911), Hartmann and Whitmore (1912), and James (1914), and there is no doubt that some of the amœbæ observed by these investigators were actually dividing forms, although Dobell (1919) regards all of the forms described as dividing organisms to be degenerative in character.

The type of nuclear division in the vegetative forms of *Endamœba coli* has been the cause of much argument among protozoologists. Schaudinn (1903) regarded it as amitotic, while others considered it mitotic, or partaking of the characters of both types of division. In 1911 I described the process of division in this species of amœba as I had observed it in living organisms. The nucleus elongated and small masses of bright, refractile chromatin granules appeared at the poles of the nucleus. Finally a constriction appeared at the centre of the nucleus and it divided into two, followed by the division of the body of the parasite into two practically equal portions. These observations were made upon specimens of amœba in the fæces, which were kept under a microscope placed in an incubator designed for such observations and the temperature maintained at 37° C. (98.6° F.). The process of division was only observed in a very few of the amœbæ and only after a long search and continued observation of the parasites, sometimes for several hours. These forms were certainly not undergoing degeneration, for the amœbæ resulting from the division were motile and perfectly normal in morphology. While these observations do not prove that the division of the nucleus is mitotic, they are suggestive of such a process, and Kofoid and Swezy (1922) state that the division of the nucleus in the vegetative forms, or trophozoites, is mitotic in

character, and that they have been able to demonstrate that this species always possesses six chromosomes.

Multiple fission, or schizogony, has been described as occurring in the trophozoites of *Endamæba coli* by Schaudinn (1903), Craig (1905), and others, but such a form of multiplication is now known not to occur in the trophozoites. The forms that I believed to be schizogonic were probably degeneration forms in which the collections of refractive material in the cytoplasm simulated nuclei and the fragmentation of the amœbæ resembled the division of the parasite into several small amœbæ. All recent observers agree that multiple fission does not occur in the trophozoites of this species.

In the cysts multiplication of the nucleus occurs by multiple fission, the original nucleus dividing into two, these again into two, and these again into two, thus producing eight nuclei in the fully developed cyst. It is probable that the division of the nucleus in the cysts is mitotic in character, and Dobell (1919) states that a spindle is formed and "sometimes definite chromosomes appear to be present," but at the time of writing he was not convinced of the occurrence of chromosomes at all stages.

There is no evidence that conjugation occurs in this species or any sexual method of reproduction.

**Geographical Distribution.**—The geographical distribution of *Endamæba coli* is world-wide. This parasite has been found in a large percentage of individuals in every region in which surveys have been made as to its presence. Like other intestinal parasites, it is more common in the tropics and subtropics than in temperate climates, due, in all probability, entirely to the greater facilities offered for infection in tropical and subtropical regions, where the sanitary disposal of human excreta is often neglected.

**Incidence of Infection.**—The incidence of infection with this parasite varies considerably in different localities, but it occurs much more frequently than *Endamæba histolytica*, or the other intestinal amœbæ, as shown by the reports of all observers who have investigated the subject.

Prior to Schaudinn's (1903) researches, several investigators had reported the presence of amœbæ in the stools of healthy and diseased individuals, but the species concerned had not been clearly identified. Schaudinn identified the amœba occurring in healthy individuals and in those suffering from non-dysenteric diseases, as *Endamæba coli*, and determined that in West Prussia 50 per cent. of healthy men among the farming population showed this parasite in their fæces, while in Berlin only 20 per cent. were found infected. He also examined 385 individuals living along the shores of the Adriatic Sea and found 256, or 66 per cent., of them infected with this amœba.

In 1905, I published the results of the examination of over 200 soldiers of the United States Army, coming from all parts of the United States, and found 65 per cent. of them infected with *Endamæba coli*. These men were on duty in a general hospital, and as many as eight examinations were made in many of them, thus accounting for the high rate of infection found, for Dobell (1919) and others have shown that one examination of the stools results in the demonstration of only about one-third of the actual infections in examinations for amœbæ, and the greater the number of examinations, within certain limits, the greater will be the number of positive findings.

My results were confirmed by Vedder (1906), who examined fifty American soldiers and fifty Filipino scouts, in the Philippines, the soldiers showing 50 per cent. of infection with *Endamæba coli*, while 75 per cent. of the Filipinos were infected with this parasite. In a later series of examinations, made in the Philippine Islands, Ashburn and I found seventy-six infections with this amœba in 107 American soldiers serving at the Division Hospital, in Manila, or 71 per cent. As a result of our observations we concluded that a large percentage of American soldiers were infected with this parasite in the Philippines, but that this infection was not accompanied by any symptoms of disease.

The observations of Schaudinn, Craig, Vedder, and Ashburn and Craig were the first in which *Endamæba coli* was actually identified as the species present, but since they were made a great deal of data has been published regarding this subject, contributed chiefly through the extensive surveys on intestinal parasites in troops made during the World War and afterward. Wenyon (1916) examined the stools of 556 British soldiers from the Mediterranean area and found 39 per cent. infected with *Endamæba coli*, while Carter, Mackinnon, Mathews, and Smith (1917) examined the stools of 4,068 British soldiers convalescent from dysenteric and other diseases, from the same area, and found that 29.7 per cent. showed infection with this parasite, an average of three examinations being made in each case.

Kofoed and his co-workers (1920) found 473 infections with *Endamæba coli* in 2,300 American soldiers who had overseas service, or 20.5 per cent., while in 576 having only home service, only 92 were infected, or 15.9 per cent. Only one examination was made in these cases. Jepps (1921) examined 971 British soldiers in hospital at Southampton and found 295 infected with this amœba, while Dobell (1921), in his compilation of data relating to the rate of infection in natives of England who had not been abroad, states that in 3,146 individuals examined, 18.1 per cent. were found infected with *Endamæba coli*.

The latest published observations are those of Boeck (1923), who records the examination of the stools of 8,029 individuals from all parts

of the United States. Of this number, 1,583, or 19.6 per cent., were found infected with *Endamæba coli*, only one examination being made in most instances. Had three examinations been made it is probable that close to 60 per cent. would have been found positive for this parasite. In 565 individuals in an institution in whom the stools were examined six times, Boeck found 308 positive for this parasite, or 61 per cent., about the same percentage (65) which I obtained in 1905 in soldiers on duty at an army general hospital.

The data given above prove that infections with *Endamæba coli* are very numerous and that a large percentage of individuals harbor this parasite. So far as I have observed, infections with this parasite continue indefinitely, I have found the amœbæ constantly present in the stools of certain individuals for months. In one individual, whom I have examined frequently, *Endamæba coli* has been present in the stools for a period of nearly twenty years, and I believe that most infections with this parasite continue indefinitely when once firmly established.

**Method of Transmission.**—*Endamæba coli* is transmitted from man to man through food and drink contaminated with the cysts of this parasite. Infection can occur only through the mouth and only the cysts are infective. Grassi (1888), Calandruccio (1890), and Schaudinn (1903) claim to have produced infection in man by feeding material containing the cysts, and Walker and Sellards (1913) demonstrated beyond question that the transmission of this infection is through the agency of the cysts by feeding 20 volunteers material containing the cysts, of whom no less than 17 became parasitized, or 88 per cent., both motile and cystic amœbæ of this species appearing in the stools in large numbers and continuing indefinitely.

**Experimental Infection of Lower Animals.**—None of the lower animals are susceptible to infection with *Endamæba coli* so far as is known. Casagrandi and Barbagallo (1897) and Schaudinn (1903) state that they produced infection in the cat by feeding the cysts of this amœba and by rectal injections but their observations must have been erroneous as no one else has been able to succeed in infecting this animal. Quincke and Roos (1893) were unsuccessful in this respect, and in 1905 I recorded negative experiments with cats, in which I fed material containing both the cysts and trophozoites to these animals, and injected the same material rectally, with no evidence of infection occurring in any of the animals, although the experiments were repeated several times in some of them. Similar results have been obtained by others, and I am fully in agreement with Dobell (1919) when he states: "I do not believe that it is possible to infect the cat with *Endamæba coli*, or to obtain any stages of development in this host."



There is, at present, no evidence, of scientific value, that it is possible to infect any of the lower animals with *Endamæba coli*.

**Relation to Disease.**—It may be stated that the evidence is incontrovertible that *Endamæba coli* is a harmless commensal living in the intestine of man. This fact has been experimentally proven by Walker and Sellards (1913), who fed 20 volunteers with material containing the cysts of this parasite, of whom 17 became parasitized. None of the infected individuals developed any symptoms of diarrhœa or dysentery, although observed for several months, during which time their stools were positive for the amœba.

The observations of Walker and Sellards confirmed the negative results obtained previously by numerous investigators in animal experiments and definitely proved that *Endamæba coli* is not a pathogenic parasite and has nothing to do with the etiology of dysentery or any other intestinal disease.

**Prophylaxis.**—As *Endamæba coli* is transmitted from man to man in the same manner as *Endamæba histolytica*, what has been stated regarding the prophylaxis of the latter is equally applicable to this species of amœba. Prophylaxis is not important from the standpoint of health, as the parasite is harmless.

**Diagnosis.**—The diagnosis of *Endamæba coli* is considered in Chapter V, in the discussion of the differential diagnosis of the intestinal amœbæ of man.

Species III. ENDAMŒBA NANA (Wenyon and O'Connor, 1917),  
Craig, 1921.

Synonyms: *Entamæba coli*, Werner, 1912, *pro parte*. *Amæba limax*, Wenyon, 1916. *Entamæba nana*, Wenyon and O'Connor, 1917. *Endolimax intestinalis*, Kuenen and Swellengrebel, 1917. *Endolimax nana*, Kofoid and Swezy, 1917. *Vahlkampfia nana* (Wenyon and O'Connor), Brug, 1917. *Endolimax nana* (Wenyon and O'Connor, 1917), Brug, 1918.

**History and Nomenclature.**—This species of amœba is of interest because it is frequently present in the human intestine and its cysts may be confused with those of *Endamæba histolytica* by one untrained in the study of the intestinal amœbæ, as they contain four nuclei when fully developed.

The first clear description of this species was given by Wenyon and O'Connor, in 1917, and they named the parasite *Entamæba nana*. Dobell (1919) conjectures that it was seen before this by numerous observers, as Gauducheau (1908), Elmassian (1909), Koidzumi (1909), Werner (1912), Craig (1913), and James (1914), but as there is no evidence beyond his opinion that any of these observers studied this species, it is obviously impossible to state definitely who first partly described the species. As this amœba is a rather common parasite of the intestine

of man it is practically certain that every careful observer from the time of Lösch, who studied the amœbæ found in the human intestine, must have seen it, but all failed to recognize it as a distinct species. Personally I know that I noted the motile stage of this amœba as far back as 1905, but confused it with the small trophozoites of *Endamæba histolytica*, while later I regarded the 4-nucleated cysts of this species as atypical cysts of *Endamæba histolytica* or developing cysts of *Endamæba coli*. Such errors were made by practically every observer until 1913, when Wenyon studied this amœba and recognized it as a distinct species, although he did not give it a name.

The nomenclature of this amœba is not in a very satisfactory condition, and still far from agreed upon by all protozoologists. Wenyon and O'Connor (1917) called it *Entamæba nana*, while Kuenen and Swellengrebel (1917) redescribed it and called it *Endolimax intestinalis*. Brug (1917) placed the organism in the genus *Vahlkampfia*, calling it *Vahlkampfia nana*, but, in 1918, changed the name to *Endolimax nana*, thus adopting the generic name of Kuenen and Swellengrebel and the specific name of Wenyon and O'Connor. Dobell (1919) has accepted this name and most writers use it in describing the parasite.

In my opinion the placing of this amœba in a distinct genus, *Endolimax*, is not justified. The only way in which this amœba differs from the amœbæ classified in the genus *Endamæba* is in the structure of the nucleus, in which it is the form of the karyosome alone that is different from the karyosome of the endomœbæ, in that it does not occur as a solid mass, but is broken up into two or more masses united by threads of chromatin. Otherwise, in the vegetative stage, this amœba does not differ in any essential respect from the amœbæ placed in the genus *Endamæba*. The cysts of this species contain four nuclei and do not differ from the cysts of the endamœbæ except that the majority of them are oval in shape, and the karyosome of the nucleus or nuclei resembles that of the vegetative form. The differences are no greater between *Endamæba histolytica* and this species than between the former and *Endamæba coli*, and to be logical, one should place every amœba so far described in man in a separate genus if a new genus is considered necessary in the case of *Endamæba nana*. I do not believe that one is justified in accepting a mere difference in the shape of the karyosome or of the cysts as sufficient evidence upon which to establish a new genus even in such simple organisms as the amœbæ, and accordingly I cannot accept the genus *Endolimax* established by Kuenen and Swellengrebel to include *Endamæba nana*. I do believe that the difference in the structure of the nucleus of this species is sufficient upon which to base a subgenus, and I regard *Endolimax* as a subgenus of *Endamæba*, but the subgeneric name should not be used in combination at present, and the

amœba should be known as *Endamœba nana*. The classification of the parasitic amœbæ is thus simplified and this organism placed more accurately from a biological standpoint. I can see no justification for the erection of the genus *Endolimax* to include this amœba, which is so closely related morphologically, and in its life-cycle, to the amœbæ included in the genus *Endamœba*, and I believe that Wenyon and O'Connor were correct in considering that this species was cogenetic with *Endamœba histolytica* and *Endamœba coli*.

**Morphology.**—*Endamœba nana* has three stages in its life-cycle, a motile or vegetative stage, a pre-cystic stage, and a cystic stage, in each of which the morphology is different in both living and stained preparations.

1. **Vegetative or Motile Stage.**—*a. Living Specimens.* The size of the trophozoites of *Endamœba nana* is given by Dobell (1919) as varying from six to twelve microns in diameter, but specimens are seen as small as five microns in diameter and as large as sixteen microns in diameter, the average being about eight microns in diameter. These measurements apply to the living amœbæ, for in the stained amœbæ the diameter is from one to two microns smaller. As compared with either *Endamœba histolytica* or *Endamœba coli* this amœba is small, averaging less than one-half the diameter of either of the species mentioned.

The *cytoplasm* appears granular, moderately refractile, and of slight consistence. The *nucleus* is not visible save in rare instances, in the living amœba, and when visible appears as a broken mass of refractile material lying in the cytoplasm. Food vacuoles are generally present, filled with bacteria which appear as refractile dots and rods within the vacuoles. Other ingested material, as crystals, leucocytes, or red blood corpuscles, do not occur in the cytoplasm of this amœba. A contractile vacuole is not present. I have never observed red blood corpuscles in this species of amœba.

*Motility* is present in *Endamœba nana* in freshly voided fæces but it is lost rapidly upon exposure of the stools to room temperature. Motility is produced by the extrusion of pseudopodia formed by the ectoplasm. The pseudopodia are hyaline and refractile in appearance and are generally well differentiated from the endoplasm. In amœbæ that are sluggishly motile the pseudopodia are broad and blunt, and this is the type most frequently observed in the fæces. However, if the fæces are examined at once and the preparations kept at body temperature under the microscope in an incubator or upon a warm stage, the amœbæ are actively motile and the pseudopodia in such organisms are more slender and finger-like in shape and are extruded quite rapidly. In most specimens examined under ordinary conditions the motility is very sluggish and feebly progressive, but it may be quite rapid and progressive in amœbæ

observed on the warm stage. When active the trophozoites of *Endamæba nana* resemble, except in size, those of *Endamæba histolytica* very closely, and for many years were mistaken for trophozoites of the latter species in unstained preparations. Motility soon ceases after the fæces have stood at room temperature and the organism degenerates rapidly.

*b. Stained Preparations.* The staining reactions of *Endamæba nana* with the hæmatoxylin stains are similar to those of other amœbæ, the cytoplasm staining brown or bluish-gray, while the chromatin of the nucleus, the nuclear membrane, and the karyosome stains black.

The *nucleus*, in stained specimens of the vegetative form of *Endamæba nana*, is very characteristic if preparations have been made from the fæces within a few moments after passage and fixed and stained immediately. It varies in size from 1 to 3.5 microns in diameter, the average measurement being about 2 microns in diameter. The nuclear membrane stains intensely black, showing that it is rich in chromatin, but there is no uniform layer of chromatin granules lining its inner surface, as in *Endamæba histolytica* and *Endamæba coli*, although a few isolated granules of chromatin may sometimes be seen upon the inner side of the membrane. The space between the nuclear membrane and the karyosome is free from chromatin, but in very well-stained and differentiated preparations traces of a linin net-work may sometimes be seen in this space.

The *karyosome* is the characteristic portion of the nucleus in this species and it varies greatly in appearance in different organisms. This variability in the appearance of the karyosome was first emphasized by Wenyon and O'Connor (1917), and Dobell (1919) states that the variations are so great that hardly any two amœbæ are alike in this respect. In my experience many specimens of this species present nuclei in which the karyosomes are practically identical in appearance, but the vast majority of the amœbæ of this species present dissimilar karyosomes due to the variation in the arrangement of the chromatin of which they are composed.

Unlike *Endamæba histolytica* and *Endamæba coli*, the karyosome of this species does not form a compact round mass of chromatin either at the centre of the nucleus or situated eccentrically, but is divided into two or more portions connected by threads or irregular strands of chromatinic substance. One of these portions is much larger than the others and generally situated eccentrically, near the nuclear membrane, and in organisms undergoing degeneration, in contact with the latter. It is only in fresh material that the nucleus of *Endamæba nana* presents a typical appearance, as in degenerating specimens, the karyosome may be in the form of a solid, irregular mass of chromatin at one side of the nucleus and in



contact with the nuclear membrane. In typical specimens there is always a perfectly clear space between the karyosome and the nuclear membrane, but in degenerating organisms this space may contain chromatin grains. The collection of the chromatin of the karyosome at one side of the nucleus in a single mass is not the normal appearance of the nucleus, as first pointed out by Dobell, although many writers have described it as being the characteristic appearance, and Kofoed, Kornhauser, and Swezy (1919) state that one of the characteristic features of *Endamœba nana*, in the vegetative stage, is the absence of a central granule, the peripheral chromatin being massed in a single large clump at one point in the nucleus. There are so many variations in the arrangement of the karyosome in this species that it is impossible to describe them, but a clear idea of the appearance of the nucleus may be obtained by a study of the illustrations.

In stained specimens food vacuoles are observed filled with bacteria, but it is only in degenerating organisms that the entire cytoplasm appears filled with bacteria. Dobell (1919) has observed in the cytoplasm of *Endamœba nana* an organism belonging to the genus *Sphærita*, Dangeard, which is apparently a parasite of this species of amœba. In the living specimens this organism appears as a regularly arranged mass of refractile spores within what appears to be a vacuole. In stained specimens the spores stain black and measure about 0.75 micron each in diameter. The regular arrangement of the spores forms a morula-like mass and the amœbæ containing this parasite are very characteristic in appearance. This parasite has never been observed in either *Endamœba histolytica* or *Endamœba coli*.

The vegetative forms of *Endamœba nana* do not occur in formed stools and are found only in liquid stools in diarrhœal or dysenteric conditions or after a saline cathartic in normal individuals.

2. **Pre-cystic Stage.**—*a. Living Specimens.* The pre-cystic forms of this species, when unstained, appear as colorless, very refractive, oval or round bodies, of practically the same size as the trophozoites, or vegetative stage of development. The cytoplasm is free from vacuoles and bacteria, and the nucleus is generally visible as a refractive mass of chromatin lying eccentrically in the cytoplasm.

*b. Stained Preparations.* The morphology of the pre-cystic forms in stained preparations is similar to that of the vegetative forms except that bacteria are not present in the cytoplasm and vacuoles are absent. The structure of the nucleus is similar to that observed in the trophozoites.

3. **Cystic Stage.**—*a. Living Preparations.* The cysts of *Endamœba nana* are developed from the pre-cystic forms, the latter secreting a cyst wall. In the living condition the cysts appear as refractive, colorless,

oval bodies enclosed in a definite membrane, the cyst wall, and containing from one to four nuclei which are rarely well defined in the unstained cyst. Some of the cysts may be circular in shape and others slightly irregular, but the vast majority are distinctly oval.

The *size* of the cysts is given by Dobell (1919) as varying from 8 to 16 microns in length by 7 to 8 microns in breadth, while Boeck (1923) gives the diameter of the cysts as from 6 to 12 microns. The cysts are smaller than those of either *Endamæba histolytica* or *Endamæba coli* on the average, but cysts of both of these amœbæ occur which are as small as those of *Endamæba nana*, as will be noted on referring to the size of the cysts of the small races of both of these species.

In the living specimens the *nucleus* in the uninucleate cyst is sometimes definitely outlined as a refractile mass of granules, but in cysts having more than one nucleus it is very rarely that they can be distinguished in the unstained specimen. The cytoplasm may contain minute refractile granules, the nature of which is undetermined.

*b. Stained Preparations.* In stained specimens the cysts of this amœba are observed to contain from one to four nuclei. In the uninucleate cysts the structure of the nucleus is well defined in properly stained specimens, and is seen to be like that of the nucleus of the trophozoite, the nuclear membrane being free from chromatin granules on its inner surface, while the karyosome is divided into two or more parts united by delicate threads of chromatin, one portion being much larger than the others and situated eccentrically. As division of the nuclei occurs they become much smaller and in the 4-nucleated cysts the nuclei are very minute and their structure is very difficult to distinguish, although a careful examination of a well-stained specimens will show that the structure is the same, in miniature, as that of the nucleus of the trophozoite. The usual appearance of the nucleus in the multinucleate cysts is that of a round nucleus with a dimly stained nuclear membrane having at one portion of its inner surface a comparatively large mass of deep-black chromatin, the space between this mass and the membrane being unstained and perfectly clear. In very well-stained specimens the nuclear membrane is distinctly stained and the arrangement of the karyosome is similar to that observed in the trophozoites.

In specimens stained with the iodine solution some of the cysts of this species present ill-defined areas which stain dark brown and are composed of glycogen. Such cysts are rare and most of those observed contain two nuclei.

*Chromidial bodies* comparable with those occurring in the cysts of either *Endamæba histolytica* or *Endamæba coli* do not occur in the cysts of *Endamæba nana*. In some cysts irregular collections of filaments, rods, and granules occur, the nature of which Dobell regards as doubtful. It

is almost certain, I think, that these bodies are of bacterial nature, and this opinion will be shared, I believe, by any bacteriologist who will study the cysts presenting these bodies or the excellent illustrations of them by Dobell, 1919, Plate II, Figs. 28, 29.

Supernucleate cysts of this species, containing eight nuclei, have been observed by Dobell (1919), but they must be very rare as he states that he has not observed more than a dozen such cysts among the many thousands of the cysts of this species that he has examined. I have never observed a cyst of *Endamœba nana* that contained more than four nuclei.

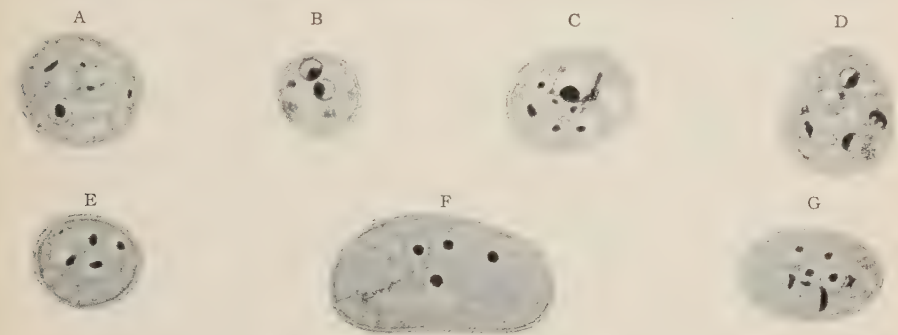


FIG. 10.—Cysts of *Endamœba nana*. (After Kofoid.) A. Uninucleate cyst containing food vacuoles with ingested material and nucleus with single mass of chromatin. B. Small spherical uninucleate cyst with typical situation of chromatin laterally upon the nuclear membrane. C. Ellipsoidal uninucleate cyst, showing typical situation of chromatin of karyosome, several food vacuoles containing ingested material, and a diffuse chromidial mass. D. Oval cyst with four nuclei. Note character of karyosome in each nucleus. E. Spheroidal cyst with four nuclei. F. Elongated oval cyst. Four nuclei, in all of which the chromatin of the karyosome appears spherical. G. Oval cyst with four nuclei and small chromidial masses. Stained with iron-haematoxylin.

**Resistance of Cysts.**—So far as I have been able to ascertain, there are no data of record as to the resistance of the cysts of this species, with the exception of the experiments of Boeck (1921), who determined that the thermal death point of the cysts was 64° C. (147.2° F.).

**Habitat.**—*Endamœba nana* is a parasite of the human intestine, but what portion of the intestinal canal it lives in has not been determined. Dobell and Jepps (1917) think that it may live in the small intestine, but it is more probable that, like the other intestinal amœbæ of man in which the exact habitat has been determined, as *Endamœba histolytica* and *Endamœba coli*, it lives in the large intestine. This species lives in the lumen of the bowel and does not invade the tissues, thus resembling *Endamœba coli*.

**Species Occurring in Lower Animals.**—This species of amœba has not been described as occurring in any of the lower animals, but amœbæ of similar morphology have been found in the leech, frog, and the flea.

**Cultivation.**—*Endamœba nana* has never been cultivated.

**Life-history.**—The life-history of this species, so far as it is known, is similar to that of the other intestinal amœbæ of man. The cysts are swallowed by man in contaminated food or drink and hatch in the in-

testine. The liberated trophozoite or trophozoites divide until conditions become unfavorable, when the pre-cystic forms are developed and finally the cysts. The latter are voided in the fæces and reach man again through contaminated food and drink, when the life-cycle outlined is repeated. The amœbæ absorb their nourishment from the material within the intestine of their host, and there is no evidence that they live upon the tissues of the intestine or obtain their nourishment from the secretions of the human body or from any body fluid.

**Method of Reproduction.**—There is very little data of record regarding the method of reproduction in *Endamæba nana*, but we know that in the vegetative stage of development the trophozoite divides by binary fission, while in the cystic stage of development four nuclei are formed within the cyst and presumably four young amœbæ are liberated at the time of excystment. Whether the division of the nucleus in this species is amitotic or mitotic has not been determined.

**Geographical Distribution.**—The geographical distribution of this species is probably world-wide. Wherever it has been searched for it has been found, and we now know that it is a common parasite of man in both temperate and tropical regions.

**Incidence of Infection.**—*Endamæba nana* is almost as common a parasite of the human intestine as is *Endamæba coli*. Dobell (1919) examined 156 British soldiers, all suffering from amœbic dysentery and positive for *Endamæba histolytica*, and found *Endamæba nana* also present in 33.3 per cent. of the men. Kofoed and his co-workers (1920) examined 2,300 American soldiers with overseas service and found *Endamæba nana* in 675, or 29.3 per cent., while in 576 home-service soldiers they found 161 infected, or 27.8 per cent. Jepps (1921), in 971 British soldiers examined in hospital at Southampton, found 278 infected with this parasite, or 28.6 per cent., while Boeck (1923), in the examinations of the stools of 8,029 individuals in the United States, found 1,060, or 13.2 per cent., showing this parasite. As an average of only one examination was made in most of these cases, and as it is admitted that only about one-third of the infections with amœbæ are discovered upon one examination, the actual number of infections with *Endamæba nana* in Boeck's material must have been over 3,100, or 39 per cent. In 505 institutional cases, Boeck found 133 infections with *Endamæba nana*, or 26.3 per cent., six examinations being made in each case. Fletcher and Jepps (1924) examined 1,034 Asiatics in the Federated Malay States and found only 2.2 per cent. infected with this parasite.

It is evident, from these figures, that *Endamæba nana*, next to *Endamæba coli*, is the most common of the amœbæ found in the intestine of man.

**Method of Transmission.**—*Endamæba nana* is transmitted from man



to man through the ingestion of food and drink contaminated with the cysts of this parasite, and what has been said regarding this subject in the consideration of the method of transmission of *Endamæba histolytica* and *Endamæba coli* is equally true of this species.

**Experimental Infection of Lower Animals.**—The only experiments indicating that any of the lower animals may be infected with *E. nana* are those of Kessel (1923), who claims to have infected rats by feeding them fæces containing the cysts of this species. It is impossible to state, at present, whether *Endamæba nana* can be experimentally transmitted to any of the lower animals or not, but it is probable that, like *Endamæba coli*, it is an obligate parasite of man.

**Relation to Disease.**—*Endamæba nana* is believed to be a harmless commensal, for there is no evidence that its presence in the intestine of man is ever followed by symptoms or lesions of disease. It occurs commonly in the stools in cases of diarrhœa and dysentery, due to other agencies, and just as commonly in the stools of healthy individuals, in this respect being exactly similar to *Endamæba coli*. While we have not, at present, the definite experimental evidence regarding its non-pathogenicity that we have in the case of the latter species, there is nothing in what we do know regarding this amœba that would indicate that it ever produces disease or is anything else than a harmless commensal in the human intestine.

**Prophylaxis.**—As this species of amœba is transmitted to man in the same manner as *Endamæba histolytica*, what has been stated regarding the prophylaxis of the latter is equally true of *Endamæba nana*. As infection with this parasite is not followed by disease, the prophylaxis of the amœba is not very important and of little practical interest from the standpoint of public health.

**Diagnosis.**—The diagnosis of *Endamæba nana* is considered in the discussion of the diagnosis of the intestinal amœbæ in Chapter V.

#### Species IV. ENDAMŒBA GINGIVALIS (Gros, 1849), Smith, Middleton, and Barrett, 1915.

Synonyms: "*Amœba gingivalis*," Gros, 1849. *Amœba buccalis*, Steinberg, 1862. *Amœba dentalis*, Grassi, 1879. *Amœba kartulisi*, Doflein, 1901. *Entamœba buccalis*, Prowazek, 1904. *Entamœba maxillaris*, Kartulis, 1906. *Entamœba gingivalis* (Gros), Brumpt, 1913. *Entamœba buccalis*, Bass and Johns, 1915. *Entamœba gingivalis* (Gros), Smith, Middleton, and Barrett, 1915. *Entamœba gingivalis* (Gros), Lynch, 1915. *Entamœba gingivalis* (*buccalis*), Craig, 1916. *Entamœba confusa*, Craig, 1916.

**History and Nomenclature.**—This was the first parasitic amœba of man discovered and, for that reason, and because for some time it was regarded as the probable cause of pyorrhœa alveolaris, it has been well studied and thoroughly and accurately described by several observers. It is a common parasite of the human mouth and was discovered by

Gros, in 1849, in the soft tartar of the teeth, and named "*Amœba gingivalis*." Steinberg, in 1862, redescribed the amœba and considered it a new species, naming it *Amœba buccalis*. The same organism was again described as a new species by Grassi (1879), who named it *Amœba dentalis*. In 1904, Prowazek gave a detailed description of an amœba occurring in the mouth, which he considered a new species and called *Entamœba buccalis*, but though Prowazek gave a better description of this amœba than his predecessors, there is no doubt that his *Entamœba buccalis* was identical with the amœba described by Gros, Steinberg, and Grassi, and it therefore follows that the specific name "*buccalis*" given by Prowazek should be replaced by the specific name "*gingivalis*" first given the parasite by Gros. In 1913, Brumpt made this emendation in the name and called the amœba *Entamœba gingivalis*, and the first to use the generic name *Endamœba* for this organism were Smith, Middleton, and Barrett, in 1915, when they called the amœba *Endamœba gingivalis*. This is the proper name of the organism, in my opinion, and has been generally accepted by American writers.

In 1916, I stated that a second species of amœba occurred in the human mouth, around the teeth, that was smaller than *Endamœba gingivalis* and differed from it in morphology, and named it *Endamœba confusa*. Further observation upon this supposed species has convinced me that the amœbæ were really the smallest forms of *Endamœba gingivalis* and that *Endamœba confusa* is synonymous with *Endamœba gingivalis*.

It is also probable that the amœbæ described by Flexner (1892) and Kartulis (1893) from the pus in abscesses of the jaw were identical with *Endamœba gingivalis*. Kartulis named the amœba he described, *Entamœba maxillaris*, in 1906, and the same amœba was named *Entamœba kartulisi* by Doflein, in 1911. Both of these names are considered synonyms of *Endamœba gingivalis* by most authorities, as they refer to a species which is believed to be identical with *gingivalis*.

Little attention was given *Endamœba gingivalis* until the appearance of a paper by Barrett (1914), in which he stated that he believed this amœba to be the cause of pyorrhœa. His work attracted the attention of others and papers were published by Chiavaro (1914), Smith and Barrett (1915), Smith, Middleton, and Barrett (1914), and Bass and Johns (1914-15), in which, while little was added to our knowledge of the morphology of the parasite, the etiological relationship of the amœba to pyorrhœa alveolaris was stressed. The authors reported excellent results with emetine in the treatment of pyorrhœa, the drug being used because of its well-known efficiency in the treatment of amœbic dysentery, and soon every prominent dentist in the country was using this drug in the treatment of pyorrhœa. At the present time very few believe that *Endamœba gingivalis* has anything to do with the causa-

tion of this disease and the "specific" treatment with emetine has been abandoned.

**Morphology.**—Unlike the intestinal amœbæ of man, *Endamœba gingivalis* has no pre-cystic or cystic stage in its life-cycle, so far as is known. While cysts of this species have been described by myself (1916) and others, I am convinced, from observations made since my description, that this amœba does not form cysts, and that the cysts that I saw, which were very few in number, were probably the cysts of some free-living amœba that happened to be present in the mouth at the time the preparations were made, as suggested by Goodrich and Moseley (1916). The pre-cystic forms of this amœba that have been described were probably immotile trophozoites. So far as is known, *Endamœba gingivalis* passes through only a vegetative stage of existence and does not form cysts.

*a. Morphology of Living Specimens.* The size of this amœba is variously given by different authorities. Gros (1849) stated that the size varies from 25 to 30 microns in diameter; Prowazek (1904) observed specimens varying in size from 6 to 32 microns in diameter. Chiavaro (1914) gives the diameter as from 5 to 20 microns; Goodey and Wellings (1916), 7.5 to 27 microns; Goodrich and Moseley (1916), 10 to 30 microns; Smith and Barrett (1915), 30 to 60 microns; and Dobell (1919), 10 to 25 microns. In my experience the size of the living immotile amœba has varied from 5 to 35 microns in diameter, the average size being from 12 to 20 microns in diameter. The very large forms, measuring from 40 to 60 microns in diameter, that have been described by some writers I am certain must be very rare, for I have never observed an amœba of this species that measured more than 35 microns in diameter in the many cases of infection with this parasite that I have studied. It is very rare to see an amœba of this species measuring more than 20 microns, and in only a very few instances have I observed organisms measuring as much as 30 microns, and still more rarely have I seen amœbæ of this species larger than this.

When immotile, *Endamœba gingivalis* is usually circular in shape, but when moving it is irregular and may be so even when motionless. The cytoplasm, when the organism is moving, is divided into a clear, hyaline outer portion, the ectoplasm, and a granular, less refractive portion, the endoplasm. In the motile amœba there is a definite and clear distinction between the ectoplasm and endoplasm, as in *Endamœba histolytica*, but the ectoplasm of this species is much less refractile than that of the former species. When motionless there is no distinction between the ectoplasm and the endoplasm.

*Motility* is well marked in individuals of this species and is progressive in character to a much greater extent than in either *Endamœba coli* or *Endamœba nana*, but not as marked as in *Endamœba histolytica*. The



pseudopodia, formed by the ectoplasm, vary much in shape. Sometimes, when motility is very pronounced, the pseudopodia are long and slender, while at other times they are very short and blunt. Often amœbæ are observed in which several pseudopodia are extruded at the same time, and when this occurs, the pseudopodia are always small and lobose in character and there is little or no progressive motion. In the vast majority of organisms the pseudopodia are short and blunt, finger-shaped pseudopodia being observed only in organisms that are very actively motile.

When the parasite is moving the endoplasm flows into the pseudopodia, the distinct division between the ectoplasmic pseudopodia and the endoplasm, which exists at the time the pseudopodia are being extruded, disappearing gradually, until, when motion ceases, there is no distinction between the ectoplasm and the endoplasm. Under high magnifications the ectoplasm appears to be finely granular in structure.

The *endoplasm* contains the nucleus, numerous food vacuoles, and ingested bodies of various kinds. No contractile vacuole is present in this species.

The *nucleus*, in the living amœbæ, is not visible, in the vast majority of instances, this species resembling, in this respect, *Endamœba histolytica*. Not infrequently an obscure body may be observed within the endoplasm which might be interpreted as a nucleus, but it is only very rarely that a definite nucleus can be demonstrated in the living organism. When it is visible it appears as a refractive ring of dots, within which may sometimes be seen one or more refractive granules.

The endoplasm contains numerous *food vacuoles*, some of them empty and some filled with ingested material. This species resembles *Endamœba coli* in the fact that its endoplasm is generally crowded with ingested material, as it is very actively phagocytic. The smallest amœbæ of this species are less vacuolated than the larger individuals, and many of the small amœbæ are apparently free from food vacuoles.

The majority of individuals of this species contain ingested material consisting usually of bacteria, and degenerated cellular material. Leucocytes and red blood corpuscles are sometimes seen, and while some authorities deny that this amœba is phagocytic for red blood corpuscles, I can testify, from personal observation, that *Endamœba gingivalis* does phagocyte red blood corpuscles, but that this occurs much more rarely than in the case of *Endamœba histolytica*. It has not been my experience that red blood corpuscles are often observed within this amœba even when the material containing the amœbæ is rich in free blood, but there is no doubt that this species can phagocyte these cells.

The most striking morphological feature in the endoplasm of this species are numerous refractile round or oval bodies within vacuoles which occur in most of the amœbæ and which sometimes almost entirely



fill the body of the parasite. These bodies are of uncertain nature, but the general consensus of opinion appears to be that they are the nuclei of cells, probably of the salivary corpuscles, as suggested by Goodey and Wellings (1916). There is no doubt that this species ingests leucocytes and the salivary corpuscles, and it is probable that the bodies mentioned are the nuclei of these cells.

*b. Stained Preparations.* The shape of *Endamæba gingivalis*, in

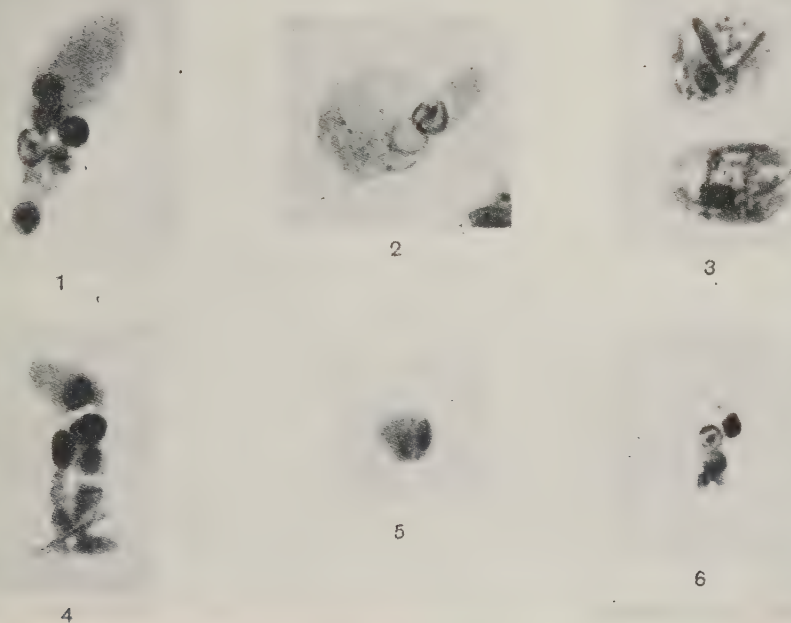


FIG. 11.—Photomicrographs of *Endamæba gingivalis*.  $\times 1,325$ . (After Goodey and Wellings.)  
 1. Typical vegetative amœba showing ingested bodies and character of nucleus. Iron-hæmatoxylin.  
 2. *Endamæba gingivalis* stained with safranin and licht-grün. 3. Vegetative amœba filled with ingested bacteria. 4. Vegetative form containing characteristic ingested bodies. 5. Very small form of *Endamæba gingivalis*. 6. Binucleate amœba, cytoplasm out of focus. 3, 4, 5 and 6 stained with iron-hæmatoxylin.

stained preparations, is usually round or oval, but many organisms are observed that are irregular in shape, fixation having occurred during the extrusion of pseudopodia. In such organisms there is a clear distinction between the ectoplasm and the endoplasm, the pseudopodium staining very faintly as compared with the endoplasm, which stains a grayish color in well-stained specimens.

The *nucleus*, when well stained, is very characteristic and can be easily distinguished from that of either *Endamæba histolytica* or *Endamæba coli*, and is smaller than the nucleus of either of these species, a fact which is very noticeable in the large amœbæ. It is also very small in

relation to the total area of the organism in the large, fully developed trophozoites. Prowazek (1904) gave the diameter of the nucleus as from 1.5 to 4.5 microns; Smith and Barrett (1915), 2 to 5 microns; and Goodey and Wellings (1916), as 2.5 to 4 microns. My experience has been that it is very rare for an individual of this species to show a nucleus exceeding 3.5 microns in diameter, and that the average diameter of the nucleus is about 3 microns.

The shape of the nucleus is usually circular, but it may be oval or elongated. The membrane stains black and the karyosome stains a dense black. The membrane is intermediate in thickness between that of *Endamæba histolytica* and *Endamæba coli*, and I cannot agree with Dobell (1919) that this membrane is so delicate that it is generally invisible. Minute grains of chromatin line the inner surface of the nuclear membrane. These grains are closely in contact and give the appearance of being an integral part of the membrane, which appears thicker than it really is because of this row of granules. The nucleus is very poor in chromatin, and in healthy organisms none of this substance can be seen within the nucleus except the small karyosome, which is usually central in position but may be eccentric. The karyosome is generally in the form of a circular dot of chromatin which is smaller than the karyosome of *Endamæba coli* but larger than that of *Endamæba histolytica*. In some amœbæ the karyosome is granular in appearance, being composed of several deeply stained, very minute grains of chromatin, but this is not the usual appearance of the karyosome. There are no chromatin grains between the karyosome and the nuclear membrane except in very rare instances, when a few very minute grains may be detected in this region. It is probable that such amœbæ are degenerating, for, in these organisms, the chromatin lining the nuclear membrane may be collected in one or more large masses instead of in the regular layer characteristic of the nucleus.

The *cytoplasm* appears granular and contains numerous vacuoles, many of them containing bacteria, the nuclei of cells, and the oval, black bodies that are so characteristic of this species. In rare instances red blood corpuscles may be observed within the cytoplasm of this amœba. The round, or oval, black bodies which are present in the cytoplasm of the vast majority of these amœbæ, in stained preparations, are evidently situated in vacuoles, as they are surrounded by a very definite unstained area. They do not occur in the very small, vegetative forms, which are generally filled with small vacuoles which do not contain ingested material, as a rule, although bacteria are sometimes observed within some of the vacuoles. As stated, the exact nature of the black-stained bodies in *Endamæba gingivalis* is still uncertain, but it is generally believed that they are the nuclei of salivary corpuscles which have been

ingested by the amœba. Whatever may be their origin, their occurrence within the cytoplasm gives this species of amœba a very characteristic appearance.

**Habitat.**—*Endamœba gingivalis* is a parasite of the mouth of man and is most apt to be present around the teeth in suppurative conditions, as pyorrhœa alveolaris. It is commonly found in the tartar of the teeth in mouths that are not kept in hygienic condition, although I have found



FIG. 12.—*Endamœba gingivalis*.  $\times 2,725$ . (After Goodrich and Moseley.) Stained with iron-haematoxylin. 1. Vegetative form of *Endamœba gingivalis* showing typical nucleus and character of the nucleus. 2, 3 and 4. *Endamœba gingivalis* showing character of nucleus and the characteristic ingested bodies. 3a, 3b and 3c. The characteristic ingested bodies of the amœba which are the nuclei of the salivary corpuscles. Remains of the cytoplasm are seen attached to the nuclei.

it present in perfectly healthy and clean mouths around the roots of healthy teeth. I have also seen amœbæ in sections of diseased tonsils that were indistinguishable morphologically from this organism and which were undoubtedly identical with it. It is especially in the pus pockets of pyorrhœa that this species of amœba is most often found, and its presence there gave rise to the belief that it was the cause of the disease.

This species may also be a parasite of some of the lower animals, as Goodrich and Moseley (1916) have found it in the pus in pyorrhœal pockets around the teeth of dogs and cats.

**Species Occurring in Lower Animals.**—With the exception of the

observations of Goodrich and Moseley, referred to above, no one has reported the occurrence of *Endamæba gingivalis* in any of the lower animals.

**Cultivation.**—In a personal letter, dated April 9, 1925, Boeck states that Drbohlay has succeeded in cultivating *Endamæba gingivalis* on the media used by Boeck and Drbohlay in the cultivation of *Endamæba histolytica*. (See Appendix.)

**Life-history.**—Very little is known regarding the life-history of this species. It is known that it lives in the mouth of man, but only the vegetative stage has been studied, and it is not known whether cysts are formed, although most authorities are convinced that such forms are never encountered in the mouth. The cysts that have been described by several investigators are now believed to have been either the cysts of free-living amœbæ or other cells which have been mistaken for cysts. I am convinced, from my own experience, that the cysts of free-living amœbæ may sometimes be demonstrated in the mouth, and the cysts that I considered, in 1916, as those of *Endamæba gingivalis* were those of such amœbæ, as I have been unable to find cysts in material containing this amœba since my first observations were published.

**Method of Reproduction.**—Reproduction occurs by simple fission, the nucleus first dividing into two, followed by the division of the cytoplasm. Chiavaro (1914) described what he regarded as mitosis in the nucleus of dividing organisms, and Nowlin (1917) also regarded the division of the nucleus as mitotic.

In my experience, reproductive forms are very rarely encountered. I have several times observed amœbæ containing two nuclei in stained preparations, but organisms in which the nucleus is evidently dividing are very seldom seen. This is the more surprising because in many infections the vegetative forms are very numerous and all sizes of the amœbæ are present, yet no reproductive forms can be demonstrated even though the cases be followed for days. In many instances preparations made at regular intervals throughout the day were negative for amœbæ showing any trace of nuclear division, and when such amœbæ were encountered they were very uncommon, and two-nucleated amœbæ were very rare.

So far as I have been able to ascertain, a primitive form of mitosis occurs in the nucleus of *Endamæba gingivalis* during division. The karyosome divides into two parts connected by a delicate thread of chromatin material, and rarely a more or less distinct nuclear spindle may be demonstrated. The nucleus becomes oval in shape and finally elongated, the two portions of the karyosome pass to the poles of the nucleus, and a constriction occurs near the centre of the nucleus which increases until the nucleus divides into two portions of equal or nearly



equal size. I have not been able to find definite chromosomes in the nucleus or to demonstrate typical mitotic figures, but I believe that the division of the nucleus is of mitotic character.

Reproduction by budding, or gemmation, in this species has been described by Prowazek (1904) and Nowlin (1917), but I am convinced that these investigators mistook degenerating amœbæ for budding organisms. Organisms are observed in stained preparations which contain granules, rods, or clumps of chromatin within the cytoplasm, while the nucleus has either disappeared or appears to be breaking up and supplying the chromatin to the cytoplasm. Sometimes the deeply stained chromatin appears to be collected near the periphery of the amœba and small projections containing some of this material may be seen apparently being budded from the periphery, but such appearances are only seen in amœbæ that are evidently degenerating and never in amœbæ that are studied in material fixed and stained at once after removal from the mouth. The same appearances were long regarded as reproductive in character in *Endamæba histolytica*, but no one, at the present time, believes that the latter species reproduces by budding.

The binucleated amœbæ always showed nuclei of about the same size, with a distinct nuclear membrane and a well-defined karyosome, situated centrally. The nuclear membrane appeared slightly thinner than in the uninucleate amœba and did not show any chromatin granules upon its inner surface. In these forms the space between the karyosome and the nuclear membrane was always free from chromatin grains or any trace of a linin net-work.

I have observed a process in living specimens of *Endamæba gingivalis* which I believe to be conjugation. Dobell (1919) states that I did not describe this process in my publication (1916), but he evidently did not read my paper carefully for I said, "However, I have observed a process which I regard as conjugation, in which two of the endamœbæ became united and there was a distinct interchange of cytoplasmic material, after which the organisms separated. This interchange of cytoplasmic substance was accompanied by very definite and marked currents within both endamœbæ, and ingested bodies within the organisms could be observed passing from one into the other." I fail to see how I could have more fully described the process and there is absolutely no doubt of its occurrence and of its being a vital phenomenon.

**Geographical Distribution.**—The geographical distribution of this species has not been accurately determined but it is probably world-wide.

**Incidence of Infection.**—*Endamæba gingivalis* is a common parasite of the mouth of man, most frequently observed in unclean mouths in the tartar of the teeth and in cases of pyorrhœa, but also found in a considerable proportion of clean and healthy mouths. The first sur-

vey to determine its incidence was conducted by Lewald (1907), but his paper appears to have been overlooked by most writers, including Dobell (1919). Lewald examined the mouths of 100 individuals and found this parasite in 71 of them, or 71 per cent., and he also determined that they occurred no matter how much care was given to the teeth. Williams (1915) found this amœba in the mouths of 30 per cent. of children with healthy gums; in 50 per cent. of children with healthy gums and carious teeth; in 84 per cent. of children showing tartar around the teeth and receding gums; and in 94 per cent. of children with spongy and bleeding gums. Mitchell, Culpepper, and Ayer (1916) found *Endamœba gingivalis* in 21.6 per cent. of children with healthy gums, and Mendel (1916) found this parasite in 57 per cent. of adults with healthy mouths and teeth. In my experience this species of amœba is generally present in cases of well-marked pyorrhœa and in the tartar of poorly-cared-for teeth, but it is also present in at least 10 per cent. of well-cared-for and healthy mouths.

**Method of Transmission.**—The method of transmission of this parasite is unknown. In the absence of cysts it would seem, as Dobell (1919) suggests, that it must be transmitted by direct contact between mouth and mouth, as the organism quickly dies upon exposure to external conditions.

**Experimental Infection of Lower Animals.**—Efforts to experimentally infect lower animals with *Endamœba gingivalis* have uniformly met with failure. Efforts have been made to produce pyorrhœa in guinea-pigs (Hecker, 1916) and in cats by injecting material containing this species into the gums, but all such efforts have resulted in failure to produce the disease or to infect the animals. At the present time there is no evidence that this species of amœba can be experimentally transmitted to any of the lower animals.

**Relation to Disease.**—A great deal of interest was awakened in this species of amœba when Barrett and Smith (1914) and Bass and Johns (1914) announced that *Endamœba gingivalis* was the cause of Riggs' disease, or pyorrhœa alveolaris. They based their assertion upon the constant occurrence of this species in pyorrhœa and its constant absence from healthy mouths. For some time their claims were accepted and, as they found that emetine apparently favorably affected the condition, the treatment of pyorrhœa with this drug became the fashion among progressive dentists. However, further researches showed that *Endamœba gingivalis* occurred in perfectly healthy mouths and in many other disease conditions in the mouth besides pyorrhœa. The observations of Chiavaro (1914), Lynch (1915), Craig (1916), Goodey and Wellings (1916), Goodrich and Moseley (1916), and many others proved conclusively that this species occurred in healthy mouths; in other con-

ditions than pyorrhœa; and that it was not present in all cases of the latter disease. In 1916, I recorded my observation upon the supposed effect of emetine upon this parasite, in which I found that the administration of this drug in cases of pyorrhœa had no effect upon the amœbæ and that throughout the period of its administration, motile and apparently healthy amœbæ were present in the lesions of the disease. In one instance there was no appreciable decrease in the number of these organisms during the administration of the drug continued for several weeks. These observations were confirmed by Goodrich and Moseley (1916) and Mendel (1916), and at the present time the treatment of pyorrhœa with emetine has been abandoned.

The evidence that has accumulated since the publication of the hypothesis of Barrett and Smith and Bass and Johns is overwhelmingly in favor of the harmlessness of *Endamæba gingivalis*, and at the present time it is generally believed that this parasite is a harmless commensal in the mouth of man. Certainly there is no evidence that supports the claim that this amœba is pathogenic that could not be equally as well used in the case of any microörganism occurring in the mouth.

In the few instances in which this parasite has been found in diseased tonsils it was evidently merely a secondary invader and there was no evidence in the sections that I have examined that the organism caused any inflammatory condition or had any cytolytic effect upon the tissues in which it was situated.

**Diagnosis.**—The diagnosis of *Endamæba gingivalis* is considered in Chapter V.

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NOTE.—In order to avoid repetition references which have been given in the list of references after Chapter II are not given in this list if referred to in the text of this chapter. If the author referred to is not found in this list of references the reader will find the reference under the proper name and date in the list of references following Chapter II.



## CHAPTER IV

THE PARASITIC AMŒBÆ OF MAN (CONTINUED). *IODAMŒBA WILLIAMSII*. *DIENTAMŒBA FRAGILIS*. PARASITIC AMŒBÆ OF UNCERTAIN OR DOUBTFUL STATUS. *COUNCILMANIA LAFLEURI*. *ENTAMŒBA PARADYSENTERIA*. *ENTAMŒBA MACROHYALINA*. *CAUDAMŒBA SINENSIS*. COPROZOIC AMŒBÆ.

THERE are two parasitic amœbæ of man that remain to be considered that are accepted as distinct species by all authorities, each belonging to a different genus. These organisms are *Iodamœba williamsi* and *Dientamœba fragilis*. In addition, there are several species that are of uncertain or doubtful status which will be described briefly, and it is also necessary to consider some of the more common and important free-living amœbæ that may be present in the stools when voided or may contaminate them afterwards, as these amœbæ have been frequently mistaken for parasitic species.

### Genus II. *IODAMŒBA* Dobell, 1919.

This genus was established by Dobell, in 1919, to include a parasitic amœba of man, *Iodamœba williamsi*, which, because of its nuclear structure and the morphology of its cysts, differs from other parasitic amœbæ. The genus *Iodamœba* is not accepted by Stiles and Boeck (1923), who place *Iodamœba williamsi* in the genus *Endolimax*, but I believe that Dobell was correct in considering that this amœba should be placed in a new genus, and I shall, therefore, accept his genus *Iodamœba* to include this parasite. As already stated, I am of the opinion that the name *Endolimax*, as the name of a genus of amœba parasitic in man, should be abandoned and that at most it should be used as the name of a subgenus of the genus *Endamœba*.

There is only one species of amœba in man belonging to the genus *Iodamœba*, the type species, *Iodamœba williamsi*.

#### Species I. *IODAMŒBA WILLIAMSII* (Prowazek, 1911). Taliaferro and Becker, 1922.

Synonyms: *Entamœba williamsi*, Prowazek, 1911. *Entamœba bütschlii*, Prowazek, 1912. "Iodine Cysts," Wenyon, 1916. *Endolimax williamsi*, Brug, 1919 (*nec* Prowazek, 1911). *Iodamœba bütschlii* (Prowazek, 1912), Dobell, 1919. *Endolimax williamsi* (Prowazek, 1911), Stiles and Boeck, 1923.

**History and Nomenclature.**—In 1911, Prowazek described an amœba occurring in the intestine of man which he regarded as a new species and named *Entamœba williamsi*. His description of this amœba con-

tained much morphological data that indicated that he was really observing *Endamæba coli*, and most authorities regarded his *Entamæba williamsi* as synonymous with *coli* until Brug (1919) announced that the so-called "iodine cysts," described by Wenyon, in 1916, were really the cysts of Prowazek's *Entamæba williamsi*. These "iodine cysts" had long puzzled protozoologists and were regarded by both Wenyon and Dobell as vegetable cells, but Brug's observations conclusively proved that they were the cysts of the amœba described partly by Prowazek, in 1911, as *Entamæba williamsi*. The status of this amœba was further complicated by the fact that Prowazek, in 1912, described another amœba which he called *Entamæba bütschlii* and which was really identical with *Entamæba williamsi*. The explanation of the resemblance in Prowazek's description of *Entamæba williamsi* to *Endamæba coli* was the fact, as proven by Brug (1921) and Nöller (1921), who examined Prowazek's original slides, that they contained both *Endamæba coli* and *Entamæba williamsi*, and that he confused the two species in his description. Prowazek described this amœba in part only, but the specific name "*williamsi*," which he gave the organism, is valid and should be retained for this organism. Brug (1919) considered that the amœba should be placed in the genus *Endolimax* and named it *Endolimax williamsi*, but this species is certainly far removed in morphology from *Endamæba nana*, which Brug considered the type species of the genus *Endolimax*.

Dobell (1919) named this species *Iodamæba bütschlii*, as he regarded Prowazek's description of the organism, which he named *Entamæba bütschlii*, as the first clear description of the species. However, as pointed out by Taliaferro and Becker (1922), the fact that Brug (1921) has designated Prowazek's original slides containing his *Entamæba williamsi* as the type material of the species, and the identification of this material with the amœba of the "iodine cysts" by Brug (1921) and Nöller (1921), renders the name *williamsi* the valid name of this amœba. Taliaferro and Becker (1922) accept Dobell's genus *Iodamæba* and have named this species *Iodamæba williamsi* (Prowazek, 1911), which I believe to be the correct name of the organism.

The reader is referred to the articles by Taliaferro and Becker (1922) and Stiles and Boeck (1923) for a full discussion of the nomenclature of this species.

**Morphology.**—*Iodamæba williamsi* possesses a vegetative and cystic stage of development in each of which the morphology of the species is characteristic.

1. **Vegetative Stage.**—*a. Living Specimens.* The size of the resting trophozoite of *Iodamæba williamsi* in the living condition varies considerably. Kuenen and Swellengrebel (1917) gave the diameter as from 10 to 12 microns; Dobell (1919), as from 5 to 20 microns, the average

being about 9 to 13 microns; Brug (1921), 7 to 20 microns; and Taliaferro and Becker (1922) found that the diameter of stained specimens varied from 9 to 14 microns, the usual diameter being about 11 microns. The largest specimen that they saw measured 20 by 15 microns.

The general morphology of the living vegetative form is very like that of *Endamæba coli*. Motility is usually sluggish and there is no sharp distinction between the ectoplasm and the endoplasm when the amœba is moving. The pseudopodia, formed by the ectoplasm, are broad and rounded, and appear finely granular under high magnifications. Motility is slowly progressive when the organisms are examined in freshly voided stools, but they quickly become motionless at room temperature. Pseudopodia are often extruded when the amœba is motionless, and at such times the pseudopodia appear hyaline and more refractile than the endoplasm and can be easily distinguished.

The *nucleus* is usually invisible, unlike the nucleus of *Endamæba coli*, but sometimes it can be seen, appearing as a refractile circle enclosing a large refractile central karyosome.

The *cytoplasm* usually contains food vacuoles which may be very numerous or few in number. Bacteria, crystals, vegetable cells, and granular débris may be present in the vacuoles, but red blood corpuscles never occur within the cytoplasm of this species. In some amœbæ, which are probably degenerating, the cytoplasm contains refractile granular masses which have been regarded by some authors as evidences of schizogony, but that such a process occurs in this species has been disproven by the observations of Dobell (1919) and Taliaferro and Becker (1922). A contractile vacuole does not occur in this species of amœba, the organism, in this respect, agreeing with all the other parasitic amœbæ that have been described.

*b. Stained Preparations.* In stained preparations the characteristic feature of *Iodamæba williamsi* is the structure of the nucleus, which differs greatly from that of any other of the parasitic amœbæ of man, more closely resembling that of some of the free-living amœba. It is best studied in hæmatoxylin-stained specimens and is very easily differentiated from the nucleus of any of the amœbæ that have been described.

In size the *nucleus* is about one-quarter the diameter of the amœba in which it is situated. The *nuclear membrane* is well stained and thicker than that of *Endamæba histolytica*, and the inner surface sometimes shows small granules of chromatin upon it, which, in degenerating amœbæ, may be collected into irregular masses. The *karyosome* is large and situated in the centre of the nucleus, its diameter being about one-half that of the diameter of the nucleus. In well-stained preparations the centre of the karyosome is lighter in color than the remainder, and

some writers have regarded this area as a centriole, but this interpretation is not accepted by Dobell (1919) or Taliaferro and Becker (1922).

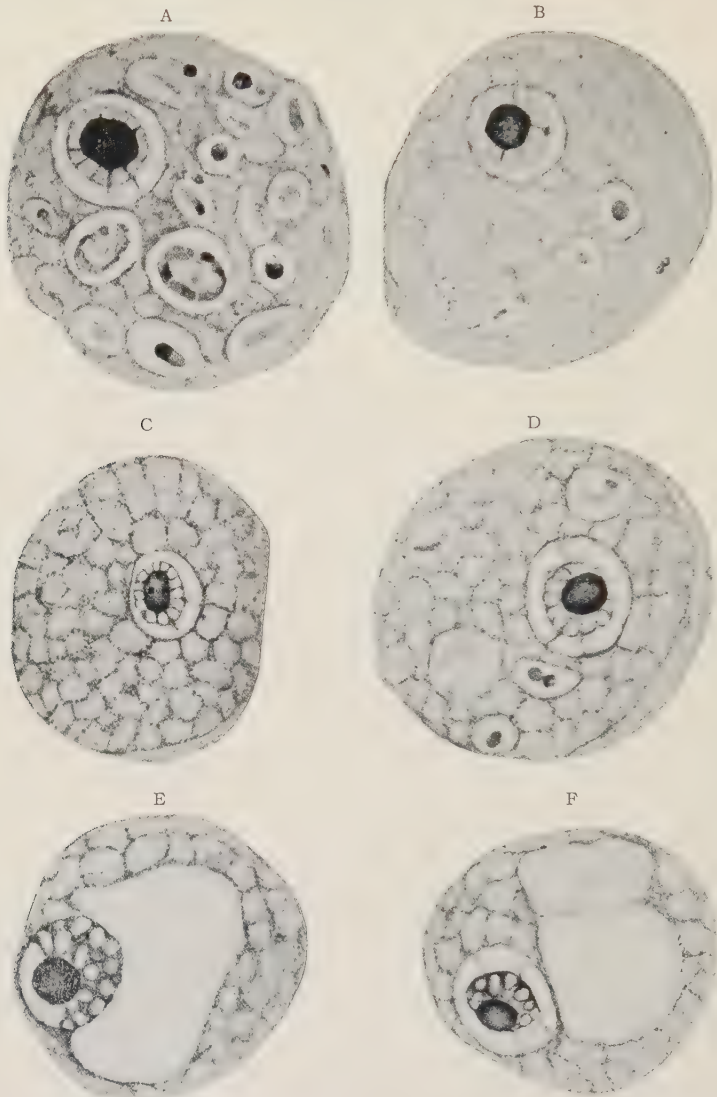


FIG. 13.—*Iodameba williamsi*.  $\times 4,300$ . (After Taliaferro and Becker.) A and B. Active amebæ stained with iron-hæmatoxylin and eosin. The peripheral granules of the nucleus appear as a segmented ring surrounding the karyosome. C. Pre-cystic ameba stained with Mann's stain. The peripheral granules surrounding the karyosome are very clearly shown. D. Active amebæ stained with Mann's stain. Note the linin strands running from the karyosome to the nuclear membrane. E and F. Cysts stained with Mann's stain. Note the change in position of the karyosome and peripheral chromatin granules. The glycogen masses have been dissolved, leaving the clear vacuoles shown in the cytoplasm.

Surrounding the karyosome and in contact with it is a layer of large granules which are called "peripheral chromatin." These granules are



arranged in a single layer which is divided into minute areas by delicate filaments, and in well-stained and differentiated specimens it will be seen that each granule is ovoid in shape and embedded in a deeper staining substance. The appearance of this layer differs with the amount of extraction of the stain, as shown by Taliaferro and Becker (1922). If little decolorization is employed this layer will appear as deeply stained as the karyosome, or, if decolorization is carried far enough, the granules will appear colorless, while the material in which they lie will be stained. If decolorization is carried too far, the entire layer will be colorless while the karyosome is still well stained.

Between the layer of "peripheral chromatin" surrounding the nucleus, and the nuclear membrane, it is sometimes possible to distinguish a few linen threads which extend from this layer to the nuclear membrane, but usually the space between the membrane and the layer of granules surrounding the karyosome is perfectly clear and unstained. In over-stained specimens the layer of chromatin granules around the karyosome is stained as intensely as the karyosome, and in such specimens the karyosome appears much larger and may be irregular in shape.

Aside from the structure of the nucleus there is nothing characteristic about the stained specimens of *Iodamæba williamsi*. Food vacuoles are present which contain stained bacteria or other material, but no red blood corpuscles have ever been observed in either living or stained specimens of this amœba.

2. **Cystic Stage.**—*a. Living Specimens.* Before encystment this species does not decrease in size, but the amœbæ rid themselves of all ingested material and in the living condition appear as refractive, hyaline bodies, having a very sluggish motion or motionless. In stained preparations the nucleus is larger than in the vegetative forms and the amount of chromatin within it is increased, the area between the karyosome and the nuclear membrane being filled with granules of chromatin arranged in several layers. A cyst wall is formed as soon as the increase in the size of the nucleus has occurred.

The cysts of this species are most characteristic and were long known as "iodine cysts" because of their staining with iodine due to the large glycogen mass which they may contain. The cysts were first described by Wenyon, in 1915, who regarded them as of vegetable nature, an opinion shared by Dobell, while Brug (1917) considered them a new parasite. In 1919, Brug identified these cysts as the cysts of *Iodamæba williamsi*, and his observations have been confirmed by all recent workers.

The *shape* of the cysts varies greatly. Some may be round or oval while others are most irregular in shape, being fusiform, rhomboidal, triangular, square, or ellipsoidal. This great variation in the shape of the

cysts of this species is a most valuable diagnostic feature and renders their recognition very easy.

The *size* of the cysts also varies, some being very small, while others are much larger. Dobell (1919) states that the spherical or oval cysts measure from 9 to 12 microns in diameter. The measurement of the irregular cysts is difficult, but Dobell (1919) suggests that if the greatest length and the greatest breadth of such cysts be measured and the mean taken as the "size" of the cyst, an approximate measurement may be obtained. This suggestion has been acted upon by later observers, and Taliaferro and Becker (1922) state that, using this measure of "size," they have found that most cysts of this species measure from 6.4 to 16.6 microns and that the average is about 9.1 microns. Dobell (1919) found that the average "size" was between 10 and 11 microns, but that all sizes from 6 to 16 microns occurred.

In the living condition the cysts have a clear white color and are surrounded by a rather thick cyst wall having a double outline. The cytoplasm appears finely granular and contains a variable number of refractile granules which resemble cocci and which Dobell believes to be volutin. Besides these granules the cysts show a more or less circular area of less refraction than the rest of the cytoplasm, which stains a deep mahogany color with iodine and is the so-called glycogen mass, or "iodophilic body" of the "I cysts" of Wenyon. The nucleus is visible in the iodine-stained preparations, but its structure is best studied in hæmatoxylin-stained preparations.

The glycogen mass in the cysts of *Iodamoeba williamsi* is very characteristic, for while a glycogen mass occurs in the cysts of both *Endamoeba histolytica* and *Endamoeba coli*, it is not so uniformly present in the latter species nor is it so marked as regards size and intensity of staining with iodine. Not all cysts of this species show the glycogen mass, and it may be small, but usually this mass is well marked. The shape varies, but it is usually rounded or oval, and there may be more than one glycogen mass in a cyst.

*b. Stained Preparations.* The cysts of *Iodamoeba williamsi* contain a single nucleus, which differentiates this species from the other parasitic amoebæ of man. The nucleus, in stained specimens, is very characteristic and quite different in structure from the nucleus of the motile trophozoites. The nuclear membrane is usually well stained, and the nucleus often appears to lie within a vacuole in the cytoplasm, owing to the retraction of the cytoplasm surrounding the nucleus during fixation and staining. The karyosome of the nucleus in the cyst, instead of being situated in the centre of the nucleus, is situated eccentrically and in contact with the nuclear membrane, in the mature cyst. In well-

stained specimens the karyosome is seen to be composed of numerous dark-brown, or almost black, grains, among which there may be some isolated granules that stain more intensely black. The situation of the karyosome, at one side of the nucleus in contact with the nuclear membrane, gives the nucleus the so-called "signet-ring" appearance. In the younger cysts the karyosome may be situated only slightly to one side of the centre of the nucleus and not in contact with the nuclear membrane.

The layer of granules which surrounds the karyosome (peripheral chromatin) in the motile amœbæ is present in the cyst, but instead of being composed of a single layer surrounding the karyosome, these granules may be arranged in several layers and may entirely fill the nucleus upon one side. In other cysts the granules may be very few in number and in contact with one side of the karyosome. The nucleus of the fully developed cyst of *Iodamœba williamsi* resembles somewhat that of *Endamœba nana*, but to any one who has carefully studied the two organisms the resemblance is only superficial and they are easily distinguished.

As stated, the vast majority of the cysts of this species are uninucleate, but cysts having two nuclei are sometimes observed. Such cysts are regarded by Dobell (1919) and Taliaferro and Becker (1922) as abnormal. Regarding their frequency, the latter observers found only four cysts containing two nuclei among 2,000 consecutive cysts that they examined, or 0.2 per cent.

The cytoplasm of the cysts in hæmatoxylin-stained specimens contain one or more vacuoles which represent the glycogen masses which stain with iodine. In the young cysts such vacuoles are small, but in the mature cysts one or two very large vacuoles may be present which often appear to crowd the nucleus to one side of the cyst. The cytoplasm has a very marked alveolar structure and often appears retracted from the cyst wall, which is unstained and very distinct. This retraction of the cytoplasm is undoubtedly due to fixation and staining.

**Habitat.**—The exact portion of the human intestine inhabited by *Iodamœba williamsi* is not known, but it is believed to be a parasite of the large intestine.

**Species Occurring in Lower Animals.**—O'Connor (1920) and Nöller (1921) have studied an amœba parasitic in the pig, which belongs to the genus *Iodamœba*, and which O'Connor has named *Iodamœba suis*. This parasite is morphologically indistinguishable from *I. williamsi* and Nöller believes it to be identical with it and that man derives his infection from the pig. Brug (1921) has described another amœba belonging to this genus in monkeys, and has named it *Iodamœba kueneni*, and a similar amœba has been found in a Brazilian monkey by Hegner and

Taliaferro (1924), but this species is stated to be slightly different in morphology from *I. williamsi*.

**Cultivation.**—This amoeba has not been cultivated *in vitro*.

**Life-history.**—Very little is known regarding the life-history of *Iodamoeba williamsi*. The motile stage divides in the intestine by binary fission and forms cysts which do not undergo any development after leaving the body in the faeces unless swallowed by man. It is supposed that the cysts hatch in the intestinal canal, liberating a single amoeba, which repeats the simple cycle indicated.

**Method of Reproduction.**—The exact method of reproduction is unknown. Whether the division of the nucleus in the trophozoite is amitotic or mitotic has not been ascertained and no one has described the process of nuclear division exactly.

**Geographical Distribution.**—*Iodamoeba williamsi* has been found in the South Seas by Prowazek (1912), the Dutch East Indies by Kuenen and Swellengrebel and others, in Europe by Dobell (1919) and others, and in the United States by Taliaferro and Becker (1922) and other observers. It is probable that it has a world-wide distribution.

**Incidence of Infection.**—This amoeba occurs rather rarely in comparison with *Endamoeba histolytica*, *Endamoeba coli*, or *Endamoeba nana*. Wenyon (1916) found it in 29 of 556 cases of dysentery, or 5.2 per cent.; Dobell (1919) states that it occurs in about 5 per cent. of cases of dysentery; Turner and Turner (1919), in their examination of 3,277 British soldiers, found 2.3 per cent. infected with this parasite; and Brug (1920) found 12 per cent. of Europeans and 8 per cent. of natives infected in the Dutch East Indies. Dobell (1921), in a compilation of the examinations of 3,146 natives of England, who had never been abroad, found only 0.25 infected with *Iodamoeba williamsi*, while Jepps (1921), in her examination of 971 British soldiers in hospital at Southampton, found 30 infections, or 3.1 per cent.

Boeck (1923) in the examination of the faeces of 8,029 individuals from all parts of the United States, found this amoeba in 404, or 5 per cent., an average of only one examination being made in each case. In 505 institutional cases he found it in 46 cases, or 9.1 per cent., six examinations being made in each case. Fletcher and Jepps (1924) examined 1,034 Asiatics in the Federated Malay States and found only 4 infections, or 0.4 per cent. infected with this parasite.

In my experience *Iodamoeba williamsi* has been a very rare parasite, and I believe that it is safe to estimate that not over 4 or 5 per cent. of individuals are infected under ordinary conditions. It is more common in the tropics, apparently, than in temperate regions, in common with all intestinal amoebæ.



**Method of Transmission.**—The parasite is undoubtedly transmitted from man to man through food contaminated with the cysts.

**Experimental Infection of Lower Animals.**—Kessel (1923) claims to have infected rats with *Iodamœba williamsi* by feeding them material containing the cysts of this parasite.

**Relation to Disease.**—There is no evidence that *Iodamœba williamsi* is a pathogenic organism. It occurs frequently in association with *Endamœba histolytica* in cases of dysentery, but I believe that this is merely a coincidence.

**Prophylaxis.**—What has been said regarding the prophylaxis of the other intestinal amœbæ applies equally to this species, but its prophylaxis is not important as it is not a pathogenic parasite.

### Genus III. DIENTAMŒBA Jepps and Dobell, 1918.

The genus *Dientamœba* was established by Jepps and Dobell, in 1918, to include a new species of amœba parasitic in the intestine of man, which they named *Dientamœba fragilis*. This amœba is the type species of the genus and the only species belonging to this genus that has been described.

#### Species I. DIENTAMŒBA FRAGILIS Jepps and Dobell, 1918.

**History and Nomenclature.**—*Dientamœba fragilis*, according to Dobell (1919), was discovered by Wenyon, in 1909, but he did not describe it. Jepps and Dobell (1918) rediscovered the parasite and published a description of it, placing it in a new genus, *Dientamœba*, and naming it *Dientamœba fragilis*. Their observations regarding this amœba and its claim to specific distinction have been confirmed by other investigators, and there is no doubt that *Dientamœba fragilis* is a valid species, and the genus *Dientamœba* justified by the peculiar morphology of the organism, which differs markedly from that of any other amœba of man.

Cases of infection with this amœba have been recorded by Jepps and Dobell (1918), Bijlsma (1919), Kofoed, Kornhauser, and Plate (1919), Haughwout and Horrilleno (1920), Nöller (1921), Jepps (1921), Thompson and Robertson (1923), and Taliaferro and Becker (1924). According to the latter authors, about 33 cases of infection with this parasite had been recorded in the literature up to 1924.

**Morphology.**—*Dientamœba fragilis*, so far as known, does not form cysts, and only the vegetative stage, or trophozoite, of this amœba has been described. The description which follows is compiled largely from that of Dobell (1919) and Taliaferro and Becker (1924), as my experience with this parasite has been limited.

*Living Specimens.* In the living condition the size of this amœba

varies from 3.5 to 12 microns in diameter, according to Dobell (1919), the average diameter being about 8.9 microns.

*Motility* is active, and there is a clear distinction between the ectoplasm and the endoplasm in the motile organisms. The pseudopodia, formed by the ectoplasm, are flattened, leaf-like, and lobed and indented, and are hyaline in appearance. Motility is progressive in character and more marked than in either *Endamæba coli* or *Endamæba nana*.

The *cytoplasm* is colorless and granular and contains food vacuoles, but a contractile vacuole is absent as in other parasitic amœbæ.

The amœba typically contains two nuclei, but in the living organism the nuclei are generally invisible.

Degeneration of *Dientamæba fragilis* occurs very quickly after the organism is voided in the fæces and in a characteristic manner. The amœbæ cease moving, become circular in shape, and the endoplasm becomes filled with vacuoles which coalesce, forming a very large vacuole which entirely fills the organism with the exception of a narrow rim of refractile cytoplasm surrounding the vacuole.

*Stained Preparations.* In stained preparations the most striking morphological feature of *Dientamæba fragilis* is the presence of two nuclei which are similar in size and structure. Dobell (1919) states that the size of the nuclei varies from 0.8 to 2.3 microns in diameter, the average measurement being about 2 microns in diameter. In specimens stained with the hæmatoxylin stains the structure of the nuclei is very characteristic. They are spherical in shape and the nuclear membrane is very delicate and stains poorly. The chromatin of the nuclei is collected at the centre, forming a rather loose karyosome, and the space between the nuclear membrane and the karyosome is unstained and free from granules of chromatin. No centriole is present in the karyosome. Slight traces of a linin net-work may rarely be seen. Isolated granules of chromatin may be present upon the inner side of the membrane, but these are observed only in very well-stained specimens. The two nuclei may be close together or widely separated and occur in the majority of the amœbæ. Uninucleate amœbæ are observed in about 20 per cent. of the organisms. Food vacuoles containing bacilli and micrococci are present, but leucocytes or red blood corpuscles are never observed in *Dientamæba fragilis*.

Dobell was not able to find any cysts of this amœba, but recently Kofoid (1923) has described the cysts of this parasite. They are smaller than the vegetative forms and contain one or two nuclei having the same structure as the nuclei of the vegetative, motile forms. Kofoid is the only observer who has described cysts as occurring in this species and his results await confirmation.

**Habitat.**—*Dientamœba fragilis* is apparently a parasite of the large intestine of man.

**Species Occurring in Lower Animals.**—This parasitic amœba has not been found in any of the lower animals, nor have amœbæ having a similar morphology been reported in any of the lower animals.

**Cultivation.**—*Dientamœba fragilis* has not been cultivated.

**Life-history.**—The life-history of this species is probably similar to that of the other parasitic amœbæ. Until the discovery of the cysts



FIG. 14.—*Dientamœba fragilis*. (After Kofoid.) A. Quiescent uninucleate amœba. B to E. Binucleate amœbæ with vacuolated protoplasm and food vacuoles. Note the broken appearance of the karyosome. F. Encysted binucleate amœba. G. Active (pre-cystic?) amœba with four nuclei and minute chromidial inclusions. H and I. Spherical cysts with a single large nucleus, large vacuoles and small chromidial mass. Stained with iron-hæmatoxylin.

by Kofoid only the vegetative stage was known. All that is known of the life-history is that the amœba lives and multiplies in the intestine of man. It occurs only in fluid or semi-fluid stools, and in formed stools the amœba cannot be found in the vegetative stage.

**Method of Reproduction.**—The exact method of reproduction is unknown. Dobell (1919) states that division forms are very rare in the stools, and all that have been observed were uninucleate amœbæ. He thinks that the amœba is binucleate when fully developed and that multiplication occurs by a simple division of the cytoplasm, thus producing two uninucleate amœbæ. During growth the nucleus in these amœbæ divides into two, thus producing the binucleate amœba. The method of reproduction within the cyst is unknown.

**Geographical Distribution.**—The geographical distribution of this spe-

cies is probably world-wide. It has been found in man in England by Jepps and Dobell (1918) and others, in Holland by Bijlsma (1919), in the Philippines by Haughwout and Horrilleno (1920), in Germany by Nöller (1921), and in the United States by Kofoed (1923) and by Taliaferro and Becker (1924). It is probable that careful search will result in the finding of this parasite in most regions in a small percentage of individuals.

**Incidence of Infection.**—Apparently *Dientamæba fragilis* is a very rare parasite of man, but, as Dobell (1919) has pointed out, the fact that it is very delicate and quickly disappears from the stools after they are voided, is perhaps the reason for so few infections being discovered. It is doubtless true that the examination of stools immediately after passage would result in the discovery of many more infections and might prove that this species is a rather common parasite in the human intestine.

**Method of Transmission.**—The method of transmission of *Dientamæba fragilis* is unknown. The apparent absence of cysts and the extreme fragility of the vegetative form, which is not found in the formed stool, render the method of transmission of the amœba a mysterious problem that has not yet been solved. It is almost impossible to believe that transmission can be by food or drink contaminated with the vegetative forms, as these forms perish almost as soon as discharged from the body, and the only explanation appears to be that some resistant form is produced which has not yet been discovered, and which is transmitted in food and drink contaminated by this form.

**Relation to Disease.**—There is no evidence that *Dientamæba fragilis* is a pathogenic parasite.

**Prophylaxis.**—As we have no knowledge of the method of transmission of this parasite, no rules governing the prophylaxis of the infection can be suggested. Fortunately, prophylaxis is of no importance, from a medical standpoint, as infection with this amœba is not attended by any symptoms or lesions of disease.

### PARASITIC AMŒBÆ OF UNCERTAIN OR DOUBTFUL STATUS

Several observers have described amœbæ which they regarded as new species, but which have been demonstrated to be identical with previously described species. The list of synonyms given in the description of the individual amœbæ in this work sufficiently indicates the new species that have been described from time to time and that have been found to be identical, in whole or in part, with the amœbæ described.

However, there remain a few so-called species in which the specific status is still uncertain or doubtful and these will now be considered.



Species I. COUNCILMANIA LAFLEURI Kofoid and Swezy, 1921.

*Councilmania lafleuri* is the name given an amœba described by Kofoid and Swezy from the stools of ten individuals under observation for varying periods from July, 1920, to June, 1921. The following description is from their original description as I have had no opportunity of studying this parasite.

The vegetative stage, or trophozoite, occurred in diarrhœal or dysenteric stools. In the living condition the ectoplasm was clear and hyaline and there was a marked distinction between it and the endoplasm when the organism was moving. Motility was active in freshly voided stools and progressive in character. The pseudopodia were extruded singly and were broad and rounded, extrusion often occurring explosively, which the authors regard as a characteristic feature of this species. In my experience, however, the explosive extrusion of the pseudopodia is often observed in *Endamœba histolytica*.

The endoplasm was vacuolated and filled with food vacuoles containing bacteria. Red blood corpuscles were frequently observed within this amœba.

In stained specimens the nucleus showed a definite nuclear membrane lined upon its inner surface with particles of chromatin. The karyosome was slightly eccentric in situation in most specimens and was circular, or angular in shape, with a clear halo surrounding it. When mitosis was beginning the halo was lost and the karyosome was broken up into separate particles. The area between the karyosome and the nuclear membrane was generally clear, although traces of a linin net-work and a few chromatin granules sometimes occurred in this area. Amœbæ with 2 nuclei were observed. The size of the trophozoite varied between 20 and 35 microns in diameter, the average size being about 28 microns in diameter. Amœbæ as large as 63 microns in diameter were observed.

According to Kofoid and Swezy, *Councilmania lafleuri* produces cysts containing 8 nuclei, thus resembling *Endamœba coli*. The precystic and cystic forms occur in formed stools. The cysts had a distinct cyst wall and contained from 1 to 8 nuclei. They varied in shape, being circular, oval, ellipsoidal, or elongated. The size of the cysts varied from 8 to 34 microns in diameter, the average being about 16.5 microns in diameter. The cyst wall was laminated and had a triple outline and was thicker than that of *Endamœba coli*. The structure of the nucleus in the cysts was very variable due to pre-mitotic changes. The nuclear membrane was very thin and generally showed a few minute chromatin granules upon its inner surface, sometimes forming a distinct layer. The space between the karyosome and the nuclear membrane stained lightly, and traces of a reticulum were sometimes observed in this area. The karyosome was central or slightly eccentric in situation and varied in

shape with the stage of mitosis, being circular, reniform, semi-circular, or spindle-shaped. Kofoid and Swezy state that there are eight definite

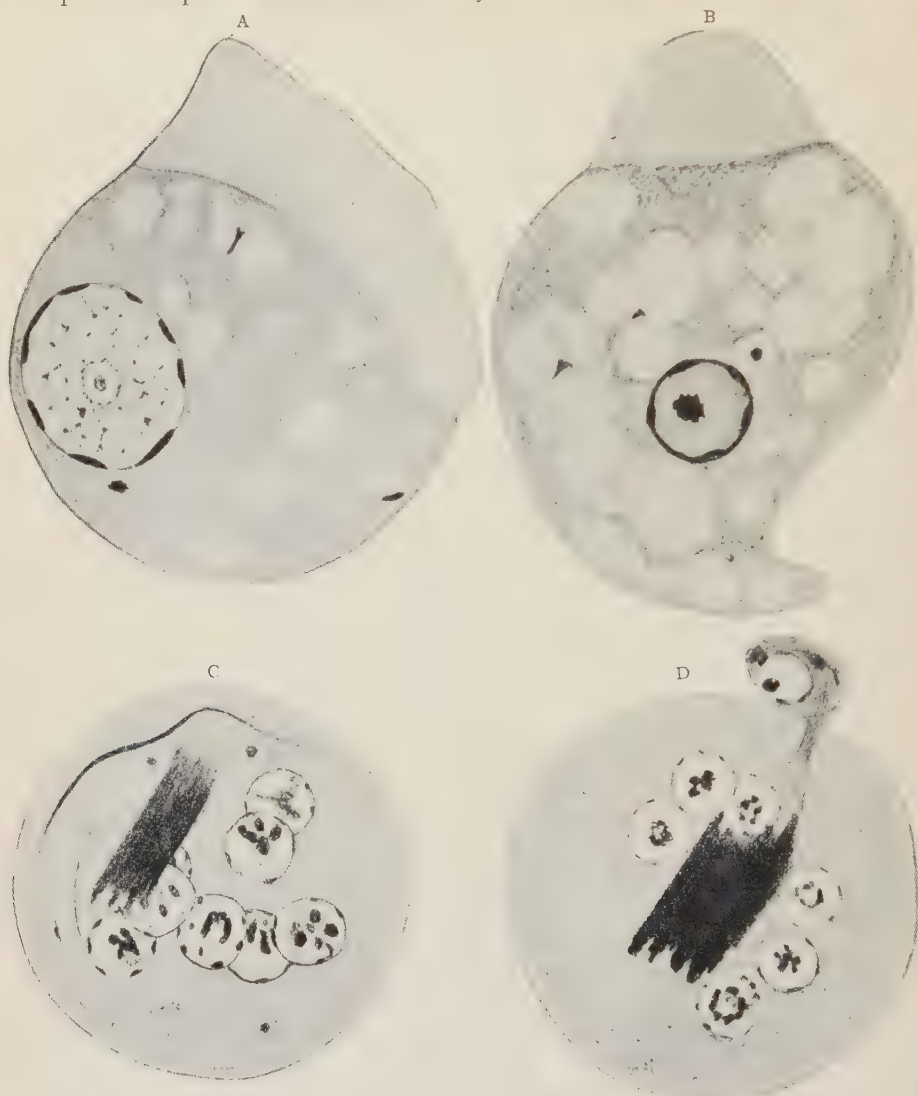


FIG. 15.—*Councilmaniana lafleuri*. (After Kofoid and Swezy.)  $\times 2,500$ . Stained with iron-haematoxylin. A. Vegetative ameba with broad hyaline pseudopodium and large nucleus with central karyosome which has been heavily decolorized. B. Vegetative ameba with clear pseudopodium. Clear distinction between ectoplasm and endoplasm. Small nucleus with ragged karyosome and heavy peripheral chromatin. C. Eight-nucleate cyst with fasciculate chromidial body with one end in beginning bud. Nuclei with dispersed karyosomes and some peripheral chromatin. D. Budding cyst with central, fasciculate chromidial body, from one end of which a chromophile area passes out into the densely chromophile cytoplasm in the pore to the chromophile bud, or amebula which contains a single nucleus, seven nuclei remaining in the cyst.

chromosomes in this species, while in *Endamoeba coli* there are but six chromosomes.

Cysts containing 1, 2, 4, or 8 nuclei were observed, but the eight-nucleated cysts were much more numerous than others.

Kofoid and Swezy (1921) describe a remarkable method of reproduction by budding in the cysts of this amœba. It is preceded by the formation of a ridge, or intra-cystic process, which causes a protrusion of cytoplasm through an opening in the cyst wall, and into this protruded portion of cytoplasm one of the nuclei of the cyst passes, followed by the detachment of the amœbula thus formed from the parent cyst. This process is repeated until the cyst has liberated 8 amœbulæ.

A similar process was described by Mathis and Mercier (1917) in the cysts of an amœba which they regarded as *Endamœba coli*, but which Kofoid and Swezy regard as undoubtedly *Councilmania lafleuri*. The authors believe that the budding process occurs in the cysts while they are still in the intestine, as indicated by the presence of budding cysts in freshly voided stools and in liquid stools after a saline cathartic.

Kofoid and Swezy believe that this species has a world-wide distribution, basing this belief upon the illustrations and descriptions of individual amœbæ by different writers. They were unable to cultivate it, but found living cysts in stools kept as long as 85 days after passage, in a glass jar at room temperature. In a personal communication, dated June 7, 1923, Professor Kofoid informs me that they have succeeded in culturing *Councilmania lafleuri* in rats and have confirmed their original description of the amœba.

As I have not studied this species I do not feel qualified to express an opinion regarding its validity. Gunn (1922) examined 8 cases which were stated by Kofoid and Swezy to be infected with this parasite, and reached the conclusion that all were infected with *Endamœba coli*, and that *Councilmania lafleuri* is identical with *Endamœba coli*. Wenyon (1922) also believes that this species is identical with *Endamœba coli*, and that the amœbæ containing red blood corpuscles were *Endamœba histolytica*, a mixed infection with these two organisms being present in some of the material studied by Kofoid and Swezy. He believes that the peculiar method of budding in the cysts was simply an accident, resulting in the rupture of the cyst and the gradual escape of its contents through the opening.

Kofoid, Swezy, and Kessel (1924) state that Wenyon's conclusions regarding this species as identical with *E. coli* are incorrect because the latter species possesses a nucleus with 6 chromosomes, while *Councilmania lafleuri* has a nucleus containing 8 chromosomes, and that, in addition, there are marked differences in the structure of the karyosomes of the two parasites. The presence of the chromophile ridges in the cysts of *C. lafleuri* and the occurrence of budding also serve to distinguish this species from *E. coli*. They found that all of the peculiar

characteristics of *C. lafleuri* were noted after transference of the species to rats. Kessel (1923) has transferred *Entamæba muris* to the genus *Councilmania*, calling it *Councilmania muris*, a classification accepted by Kofoid (1923) and Swezy (1923).

I have observed all of the morphological features noted by Kofoid and Swezy as characteristic of *Councilmania lafleuri* in amœbæ that I considered as either *Endamæba coli* or *Endamæba histolytica*, but I may have been observing *Councilmania lafleuri* in a mixed infection with the other amœbæ. Up to the present time Kofoid and Swezy's observations regarding this amœba have not been confirmed and further research is desirable.

#### Species II. ENTAMÆBA PARADYSENTERIA Chatterjee, 1920.

In 1920, Chatterjee described an amœba occurring in a fatal case of dysentery at Calcutta which he regarded as a new species and named *Entamæba paradyseutera*. This amœba was studied in material obtained at autopsy from ulcerations in the intestine, and is described as measuring from 8 to 40 microns in diameter, the average being 16 to 20 microns in diameter. There was a marked distinction between the ectoplasm and the endoplasm even in quiescent organisms, and bacteria and red blood corpuscles were present in the endoplasm as well as numerous vacuoles. The nucleus was oval in shape and stained diffusely. The karyosome was eccentric in situation and the nuclear membrane was poorly or not at all defined.

I have never seen preparations of this amœba, but I agree with Wenyon (1922) that the description of this amœba is based upon degenerating specimens of *Endamæba histolytica*. I have seen all of the morphological features that Chatterjee describes in many degenerating specimens of *Endamæba histolytica*, and the fact that he based his description upon amœbæ obtained from the lesions in the intestine three hours after death occurred is sufficient proof that the amœbæ that he described were undergoing degeneration. I do not believe that *Entamæba paradyseutera* has any claim to specific distinction.

#### Species III. ENTAMÆBA MACROHYALINA Tibaldi, 1920.

Tibaldi, in 1920, described an amœba occurring in the tonsils in two cases of tonsillitis, which he regarded as a new species and which he named *Entamæba macrohyalina*. This amœba was characterized by its large size and marked ectoplasm. The size of the amœba, as given by Tibaldi, varied from 24 to 40 microns in diameter, about twice the usual diameter of *Endamæba gingivalis*. A disproportionate amount of ectoplasm was noted in this amœba, and was considered as characteristic by Tibaldi, but this appearance may have been due to degeneration



in specimens of *Endamœba gingivalis*. There is very little in the description of this amœba by Tibaldi that would impress one with the belief that he was dealing with a new species, and I believe that he was observing atypical examples of *Endamœba gingivalis*. I have seen the latter species in exudates from the tonsils and in sections of tonsillar tissue, and many of the amœbæ appeared larger than normal and often surrounded by a definite ectoplasm. At the present time there is no reason to believe that *Entamœba macrohyalina* is a valid species.

#### Species IV. CAUDAMŒBA SINENSIS Faust, 1923.

In 1922, Faust observed an amœba in Chinese patients admitted to the Peking Union Medical College Hospital suffering from dysentery which he considered a new species and named *Caudamœba sinensis*. In two of the four patients in whom this amœba was observed there was a mixed infection with *Endamœba histolytica*.

The following description of this amœba is compiled from that of Faust, as I have not had an opportunity of studying preparations containing it:

The size of *Caudamœba sinensis* varied from 16 to 17 microns in diameter. Only the vegetative stage was observed and, although carefully searched for, no cysts were found. The cytoplasm was divided into ectoplasm and endoplasm, but the ectoplasm was only visible when the organism was moving, the pseudopodia being composed of this portion of the cytoplasm. Motility was active and progressive and, unlike any other species of entozoic amœba, the organism possessed a definite, fixed polarity, there being a definite anterior end, broadly lobose in active specimens, and a definite posterior end, which was attenuated and ended in a well-marked median caudostyle, surrounded at times by several smaller cytoplasmic projections.

In moving the endoplasm always flowed away from the caudostyle and pseudopodia were rarely observed. The nucleus, measuring from 3 to 4 microns in diameter, was situated in the anterior portion of the cytoplasm, and in stained specimens showed a definite nuclear membrane having upon its inner surface minute granules of chromatin connected with achromatic filaments. The karyosome was situated at the centre of the nucleus and consisted of a star-shaped clump of chromatin having an unstained centre.

This amœba was phagocytic for red blood corpuscles and these cells were frequently noted in the cytoplasm.

According to Faust (1923) this amœba is very susceptible to temperature, as it does not survive 40° C. Nothing is known of its life-history or method of reproduction.

The description of the amœba indicates that it is a new species para-

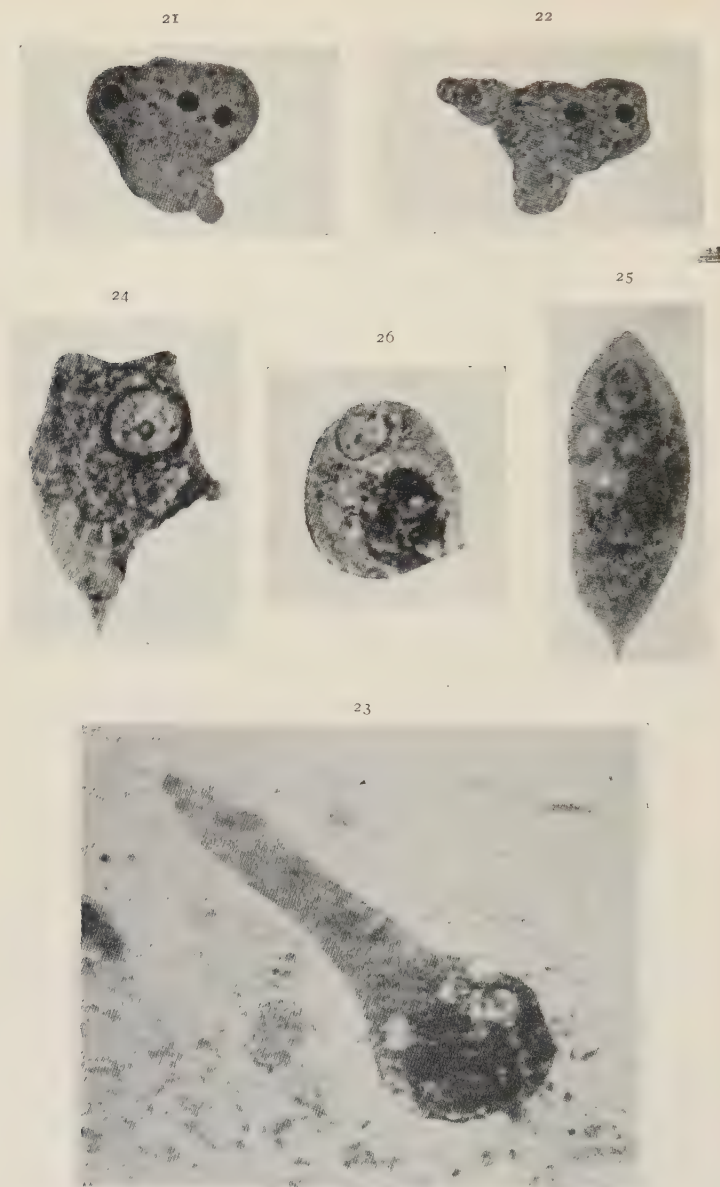


FIG. 16.—Photomicrographs of *Caudamæba sinensis*.  $\times 1,300$ . (Faust, *Journal Parasitology*.)  
 21 and 22. *Caudamæba sinensis* in active condition, showing red blood corpuscles in endoplasm. 23. *Caudamæba sinensis* in active condition. 24. Active form of *C. sinensis* showing peculiar karyosome. 25. Quiescent form of *C. sinensis* with nucleus at anterior end. Note definite caudostyle at posterior end. 26. Pre-cystic form of *C. sinensis*. Specimens stained with iron-alum hæmatoxylin.

sitic in man, and further research is desirable as Faust's observations have not been confirmed.

### Species V. ENDAMŒBA MORTINATALIUM

Smith and Weidman, 1910.

Smith and Weidman, in 1910, described an amœba occurring in the kidneys, liver, and lungs of a still-born syphilitic infant, which they named *Endamœba mortinatalium*. In 1914 the same observers described another case of infection with this amœba in which it occurred only in the lungs. This infection was in a two-months-old syphilitic child who died of pneumonia.

The amœbæ varied in size from 22 to 38 microns in diameter, and were round, oval, pyriform, or irregular in shape. A thin border of ectoplasm was sometimes visible, and short, thick pseudopodia, composed of ectoplasm, were observed in the stained specimens. The endoplasm appeared coarsely granular and vacuolated and contained chromatin-staining granules which they believed to be chromidia.

The nucleus was relatively large, measuring from one-third to one-half the diameter of the amœba, and round or oval in shape. The karyosome was large and situated in the centre of the nucleus. It was rich in chromatin and sometimes contained a centriole. The nuclear membrane was well defined, and the space between it and the karyosome generally appeared clear, although sometimes a few chromatin granules and threads were observed in this space. Reproductive forms were not observed.

The amœbæ in the kidneys and liver were always situated in small suppurative foci, while those in the lungs were contained in the air vesicles.

Smith and Weidman (1914) believe that the amœba described by them is identical with the protozoon described by Ribbert (1904) in the kidneys of a syphilitic new-born infant, and by Jesionek and Kiolemengolon (1904) in the kidneys, liver, and lungs of a syphilitic eight-months fœtus.

The observations of Smith and Weidman have not been confirmed, but deserve serious consideration and further research before they can be successfully disputed.

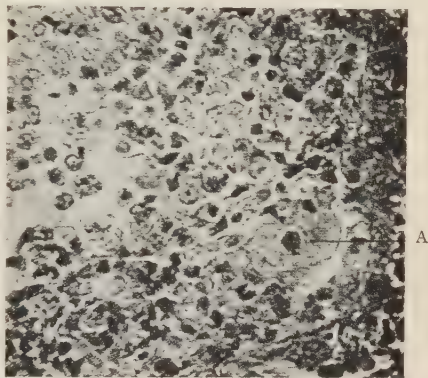


FIG. 17.—Photomicrograph of *Endamœba mortinatalium* in section of lung of syphilitic infant, stained with hæmatoxylin and eosin. (After Smith.) A. *Endamœba mortinatalium*.

## Species VI. KARYAMÆBA FALCATA Kofoid and Swezy, 1924.

This species was described by Kofoid and Swezy (1924), who found it in the feces of three individuals studied in California. Only vegetative forms were observed. In all three individuals a coincident infection with *Endamæba histolytica* was present.

They describe the parasite as being characterized by one, or rarely more, blunt pseudopodia, hyaline in appearance. The ectoplasm and endoplasm are sharply differentiated and a heavy peripheral pellicle is present which is very characteristic. The nucleus contains one or two, rarely more, large, crescentic, deeply stained masses applied to the nuclear membrane. The karyosome is spherical, situated to one side of the nucleus, and surrounded by an unstained halo, in close proximity to which a minute centriole is sometimes noted. Division of the nucleus is mitotic in character and there are approximately 20 chromosomes. Mitosis is of the type noted in the genus *Vahlkampfia*, but the authors do not believe that this amœba belongs to the latter genus or that it is coprophilic in character. Their observations concerning this new genus and species await confirmation.

## PSEUDO-AMÆBÆ

Several observers have described as new species of amœbæ various cells of the tissues or fluids of the human body or have mistaken other protozoan parasites for amœbæ. These pseudo-amœbæ include the following:

## 1. LEYDENIA GEMMIPARA Schaudinn, 1896.

This so-called amœba was discovered by Leyden and Schaudinn (1896) in the ascitic fluid from two patients suffering from malignant growths in the abdomen. Dobell (1919) believes that these "amœbæ" were really cells from the body cavity, and all of the evidence available supports his belief. There is no reason for considering "*Leydenia gemmipara*" a species of amœba.

## 2. AMÆBA MIURAI Ijima, 1898.

Ijima (1898) described amœbæ occurring in serous fluid obtained from a woman suffering from peritonitis, due to an endothelioma, to which he gave the name of *Amœba miurai*. He described it as measuring from 15 to 18 microns in diameter, spherical or oval in shape, with a pseudopodium at one end covered with cilia. The cytoplasm was finely granular and no distinction was noted between the ectoplasm and the endoplasm. From 1 to 3 nuclei were observed, but no reproductive forms were described. The amœbæ were also found in the bloody stools of the patient.

It is generally believed that Ijima mistook cells present in the serous



exudation and the stools for amœbæ, and his observations have not been confirmed. I do not believe that *Amœba miurai* can be considered a valid species.

### 3. AMŒBA PULMONALIS Artault, 1898.

This so-called amœba was discovered by Artault (1898) in the sputum, but his description was very meagre and it is impossible to state whether he was observing *Endamœba histolytica*, which might have been present in the sputum from a secondary invasion of the lungs through the rupture of a liver abscess, or whether he mistook other cells occurring in the sputum for amœbæ. The latter is more probable. At any rate, *Amœba pulmonalis* is not a valid species of amœba in the opinion of all recent observers.

### 4. ENTAMŒBA UNDULANS

Castellani, 1905.

In 1905, Castellani described an organism occurring in the fæces of patients observed in India, suffering from diarrhœa, which he considered a new species of amœba and named *Entamœba undulans*. The organism measured from 25 to 30 microns in diameter, was round or oval shape, and, unlike other amœbæ, possessed an undulating membrane which was in constant motion. A long, narrow pseudopodium was rapidly extruded from the body of the parasite at frequent intervals and quickly withdrawn. The cytoplasm was finely granular, there was no distinction between the ectoplasm and the endoplasm, and the nucleus was generally invisible. The endoplasm contained a single small vacuole which varied in position and was not contractile. Cysts were not found, and no reproduction forms were observed.

The close resemblance of this organism to certain degenerative forms of *Trichomonas hominis* suggests that Castellani might have been observing such forms of this flagellate, especially as the patients were also infected with this parasite. This opinion, first stated by myself in 1911, has been concurred in by Wenyon (1913), Hartmann (1913), and Dobell (1919), and I believe that it is now generally accepted that *Entamœba undulans* has no specific status, but is identical with *Trichomonas hominis*.

### 5. AMŒBA PYOGENES Verdun and Bruyant, 1907.

*Amœba pyogenes* was described by Verdun and Bruyant as occurring in the pus from an abscess in the malar region. Their description

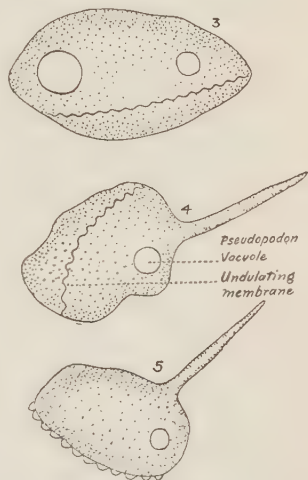


FIG. 18.—*Entamœba undulans*. (After Castellani.) 3. Organism showing undulating membrane and vacuoles. 4 and 5. Organisms showing undulating membrane, pseudopodium, and vacuole.

indicates that they were really observing *Endamæba gingivalis*, but also included the morphology of other cells which were present in the pus from the abscess. *Amæba pyogenes* is not a valid species.

### COPROZOIC AMŒBÆ

Numerous species of free-living amœbæ are found in human fæces, and these organisms have caused the greatest confusion in the description and classification of the parasitic amœbæ of man owing to the fact that they were constantly being confused with the latter or regarded as new species of amœbæ parasitic in man. Most of the amœbæ that have been cultivated from human fæces were really free-living species, although regarded by the observers who cultivated them as parasitic species. This interpretation of the amœbæ obtained in such cultures was maintained by myself for many years, but it was not until the experimental researches of Walker and Sellards (1913) that it was definitely proven that such cultivated amœbæ were free-living species and had nothing to do with the etiology of dysentery or any other disease in man. The amœbæ that have been cultivated from the pus of liver abscesses belong in the same category.

The free-living amœbæ reach the fæces either through air currents depositing the cysts upon the fæcal material or through food and drink contaminated by the cysts. When such contaminated food or drink is ingested the cysts may pass unchanged through the alimentary tract and be voided in the fæces, or, more infrequently, the cysts may hatch in the intestine and the free-motile amœbæ may be found in the stools when voided. There is no doubt that the cysts do hatch in the human intestine, under certain conditions, for I have found motile forms of free-living amœbæ in freshly voided stools that could not have been contaminated after passage, and Stiles and Boeck (1923), have also found free-living amœbæ in the vegetative stage in the stools.

Most of the free-living amœbæ found in human fæces belong to the so-called "*limax*" type, and the classification of these organisms is in a chaotic condition. Several genera have been established containing many species, but only species belonging to four genera are of interest in human pathology, *i.e.*, *Dimastigamæba*, *Hartmanella*, *Sappinia*, and *Vahlkampfi*. The species belonging to the above genera which have been found in human stools will be briefly described, as the recognition of these amœbæ is important in the differential diagnosis of free-living and parasitic species. All of these amœbæ can be easily cultivated upon Musgrave and Clegg's medium. (See Appendix.)

I. DIMASTIGAMŒBA GRUBERI (Schardinger, 1889),  
Alexeieff, 1912.

Synonyms: *Amœba gruberi*, Schardinger, 1889. *Nægleria punctata* (Dangeard), Alexeieff, 1912. *Vahlkampfia punctata* (Dangeard), Chatton and Lalung-Bonnaire, 1912. *Amœba tachypodia*, Gläser, 1912. *Nægleria gruberi* (Schardinger), Wilson, 1916. *Wasielewskia gruberi* (Schardinger), Zulueta, 1917.

This species of free-living amœba was found in the human stools by Schardinger (1889) and has since been redescribed by different ob-

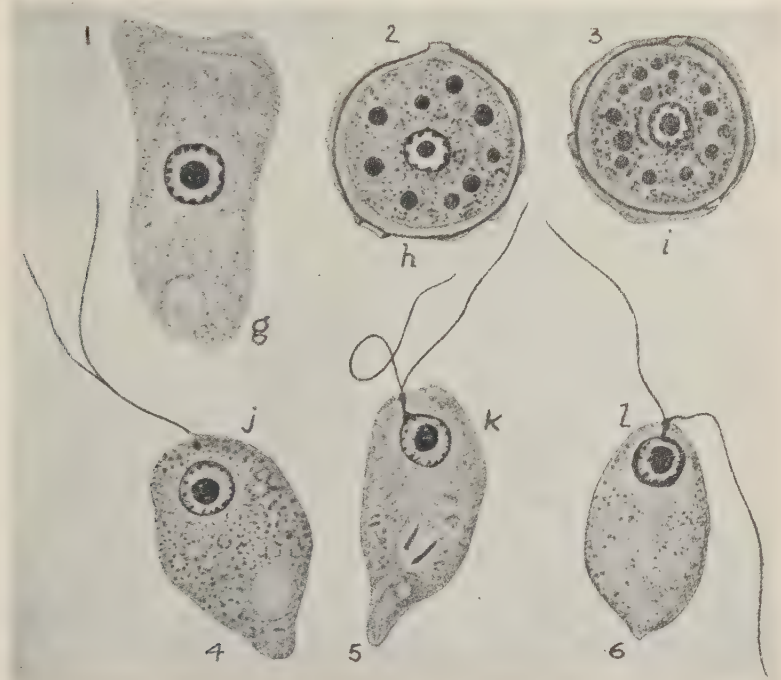


FIG. 19.—*Dimastigamœba gruberi*. (*Amœba punctata*.) (After Alexeieff.) 1. Vegetative form of amœboid stage of development. 2 and 3. Encysted stage of development. 4, 5 and 6. Flagellated stage of development.

servers and given different specific names. It is an amœba having both an amœboid and flagellate stage in its life-history. The active amœbæ measure from 6 to 18 microns in diameter, when circular in shape, with well-defined ectoplasm and endoplasm, and containing a single nucleus having a large central karyosome. The space between the karyosome and the nuclear membrane contains a few chromatin granules. There is a large contractile vacuole and the endoplasm also contains food vacuoles filled with bacteria, which form its principal food. Nuclear division is mitotic in character.

The cysts are circular in shape and measure from 6 to 12 microns in diameter. They contain a single nucleus similar in structure to the

nucleus in the motile amœbæ. The cytoplasm of the young cysts also contains circular chromidial bodies, but these disappear as the cyst becomes fully developed. The cyst wall is thick and composed of two layers and contains several pores, one of which serves as an exit for the young amœba when the cyst is placed in favorable conditions for excystation.

This amœba has a definite flagellate stage in its life-history, during which time it becomes an active flagellate. The amœbic form becomes oval in shape, and two flagella develop at the anterior end from definite basal granules, or blepharoplasts. The contractile vacuole persists and

is situated in the posterior end of the organism. These forms lose their flagella and again become amœboid organisms. This organism may eventually have to be classed with the flagellates.

The habitat of this species is in

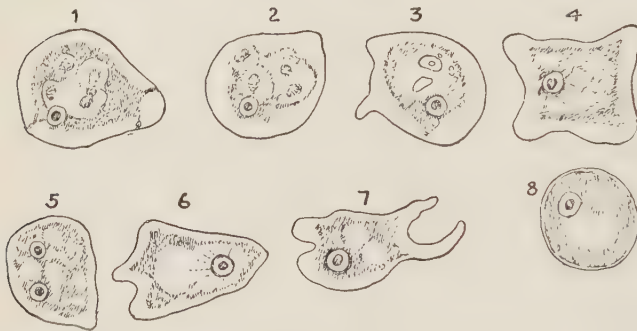


FIG. 20.—*Hartmanella hyalina*. (After Dangeard.) 1, 2, 3, 4, 6 and 7. Various forms of the vegetative, motile stage of development. 5. Binucleate form. 8. Cyst of *Hartmanella hyalina*.

water and soil and it can be easily cultivated on the Musgrave and Clegg medium. It has been repeatedly found in the human faeces, but cannot live in the intestinal tract of man or other animals.

## 2. HARTMANELLA HYALINA (Dangeard, 1900), Alexeieff, 1912.

Synonyms: *Amœba hyalina*, Dangeard, 1900.

*Hartmanella hyalina* is a free-living species of amœba that has been found in the human faeces frequently. Dobell and O'Connor (1921) believe that it is identical with the amœbæ obtained in cultures from liver-abscess pus by Wells, in India, and described by Liston and Martin (1911).

The size of the vegetative form varies from 9 to 17 microns in diameter, and its nucleus resembles that of *Dimastigamœba gruberi*. It has a single contractile vacuole and the ectoplasm and endoplasm are fairly well differentiated when the organism is moving. The nucleus divides by mitosis, and Dobell states that he has studied all stages of nuclear division in this species.

The cysts measure from 10 to 14 microns in diameter and have a cyst wall formed of two layers, the inner thin and smooth and the outer thick and wrinkled. No pores are present in the cyst wall. Chromidial



bodies are present in young cysts, but disappear later, and they are always small and circular in shape. The cysts contain only one nucleus.

This amœba has no flagellate stage of development and can be easily cultivated.

### 3. SAPPINIA DIPLOIDEA (Hartmann and Nägler, 1908), Alexeieff, 1912.

Synonyms: *Amœba diploidea*, Hartmann and Nägler, 1908. *Vahlkampfia diploidea* (Hartmann and Nägler), Calkins, 1912.

This amœba, sometimes found in human stools, is a free-living spe-

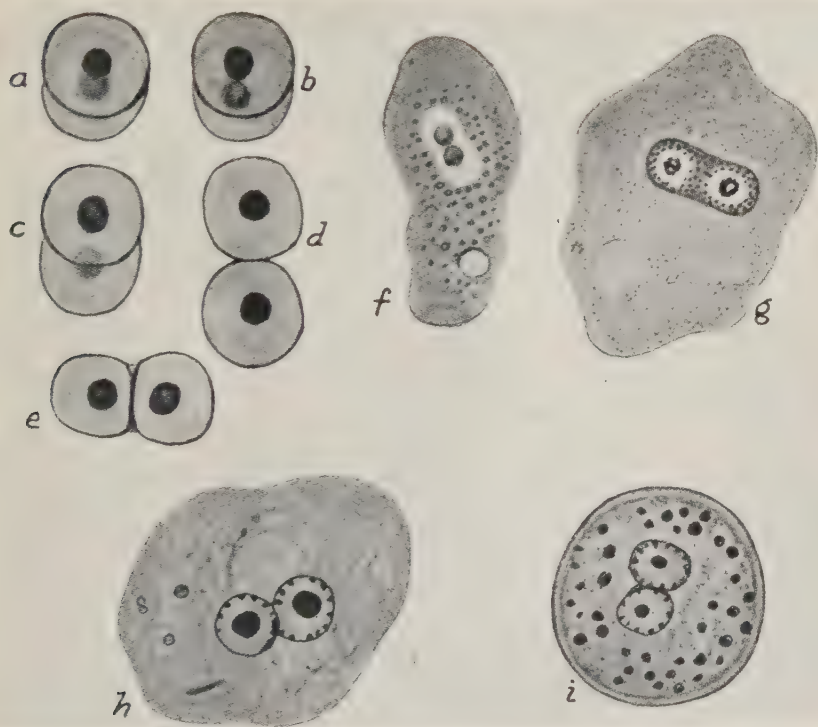


FIG. 21.—*Sappinia diploidea*. (After Alexeieff.) a to e. Various appearances of nuclei. f, g and h. Motile, or vegetative, forms of *Sappinia diploidea*. i. Cyst of *Sappinia diploidea*.

cies measuring, in the vegetative stage, from 10 to 30 microns in diameter. It is characterized by a definite cuticle, which is thick and hyaline in appearance with a smooth or rough surface. Two nuclei are present, thus resembling *Dientamoeba fragilis*, a parasitic species. The nuclei have a large central karyosome and are similar in appearance. A contractile vacuole is present in the endoplasm.

The two nuclei divide at the same time by mitosis, thus producing two amœbæ having two nuclei each.

The cysts of this species are formed after conjugation, two amœbæ joining and forming a cyst containing the two organisms. The cysts are circular in shape and measure from 10 to 18 microns in diameter. Changes occur in the two individuals within the cyst which are believed to be sexual in nature and which result in the formation of a single uninucleate amœba. When conditions are favorable the cyst hatches and the uninucleate amœba becomes free, develops two nuclei, and repeats the life-cycle. This species is easily cultivated upon the Musgrave and Clegg agar medium.

4. VAHLKAMPFIA LOBOSPINOSA (Craig, 1912), Craig, 1913.

Synonyms: *Amœba lobospinosa*, Craig, 1912. *Vahlkampfia whitmorei*, Hartmann, 1913.

This amœba was originally cultivated from human feces by Musgrave, in Manila, in 1904, and described by him as *Amœba* 11524 of his series. In 1912, I described this amœba as it appeared in cultures descended from cultures sent me by Doctor Musgrave, and named it *Amœba lobospinosa*, later, in 1913, changing the name to *Vahlkampfia lobospinosa*. Musgrave secured it in cultures from the feces of a patient suffering from amœbic dysentery and believed it to be a dysentery amœba, but it is a typical free-living species and is now known to have nothing to do with the etiology of dysentery.

The size of the organism varies, some small amœbæ in the cultures measuring as little as 8 microns in diameter, while others may measure as much as 25 microns in diameter, but the average diameter is about 16 microns. The shape, when motionless, is oval or spherical, but very irregular when the amœba is moving. Motility is sluggish and progressive motion not very marked. The pseudopodia are of two varieties, short and blunt, and long and spinose. The lobose pseudopodia are present when the amœba is moving actively, while the spinose pseudopodia are present when motility is absent or very sluggish. Both lobose and spinose pseudopodia may be present at the same time, but usually the spinose pseudopodia are present when motility is absent and are shot out quickly and almost as quickly retracted, so that it is often difficult to distinguish them. There is little distinction between the ectoplasm and the endoplasm in the small amœbæ, but in the larger amœbæ, when actively moving, the ectoplasm appears more refractile than the endoplasm.

The nucleus is well defined in the living organism, appearing as a circular, refractile body surrounded by a narrow hyaline halo, but the nuclear membrane is indistinct. In the vegetative forms there is a single contractile vacuole situated in the endoplasm which contracts once in about 15 seconds, and which, when most clearly defined, is situated near the nucleus, but travels toward the periphery before it contracts, and when it does contract is situated at the periphery.

Food vacuoles are present which are often filled with bacteria, but no cellular bodies have ever been noted in this amœba.

In stained specimens the cytoplasm stains a brownish-gray or gray with the hæmatoxylin stains and contains small vacuoles, some of which are filled with bacteria. The nucleus has a poorly stained nuclear membrane composed apparently of a rather thick layer stained black, having upon its inner surface a zone of fine chromatin granules. In many amœbæ the nuclear membrane is not distinct, but appears to merge into the cytoplasm. There is a very large central karyosome, filling at least two-thirds of the nucleus, which stains intensely. When properly differentiated the karyosome is seen to be composed of granular chromatin, and in some organisms one or more very intensely stained chromatin dots may be seen at the centre of the karyosome. The space between the karyosome and the nuclear membrane is unstained and does not contain chromatin granules or any traces of a linin net-work, appearing as a clear halo surrounding the karyosome.

In stained specimens a single large vacuole may be distinguished, in some instances, representing the contractile vacuole, the amœba having been fixed just before the vacuole contracted, but in many of the amœbæ the contractile vacuole is not visible in the stained specimen.

During the vegetative stage of existence reproduction occurs by simple fission, the nucleus often showing well-marked mitotic figures. A well-defined spindle is formed and an equatorial plate is often well developed, stretching across the spindle midway between the polar bodies and composed of two very definite rows of chromatin granules. Eventually the nucleus divides into two nuclei, followed by the division of the body of the amœba.

The cysts of *Vahlkampfia lobospinosa* are produced whenever conditions are unfavorable to vegetative life, and in cultures begin to appear within one week after the culture is started, under ordinary conditions.

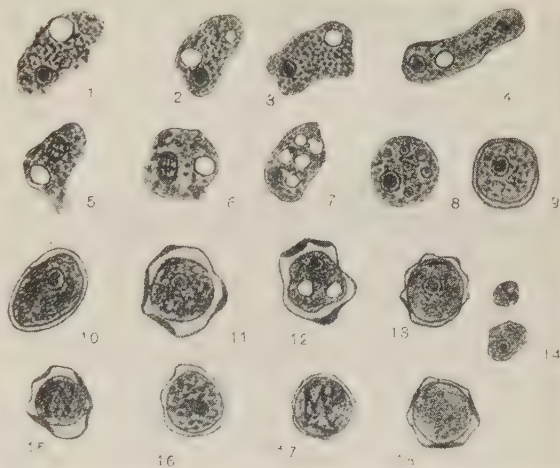


FIG. 22.—*Vahlkampfia lobospinosa*. Vegetative and cystic forms. 1 to 7. Various examples of vegetative forms showing character of nucleus and the contractile vacuole. 8 to 18. Various forms of the cyst of *Vahlkampfia lobospinosa* showing the smooth cystic wall of the young cysts and the wrinkled and folded-over cyst wall of the old cysts. 17 and 18 are degenerate cysts without a nucleus.

In size the cysts vary from 8 to 15 microns in diameter, the average being about 10 microns in diameter. The youngest cysts, when living, have a delicate refractile cyst wall, with a single outline, but the older cysts show a thick wall, very refractile in appearance, and having a well-marked double outline. In the fully developed cyst the cyst wall is composed of two layers, an inner very thin layer and an outer thick layer. The cysts are spherical in shape as long as the culture medium is moist, but when it becomes dry they contract unequally and irregular

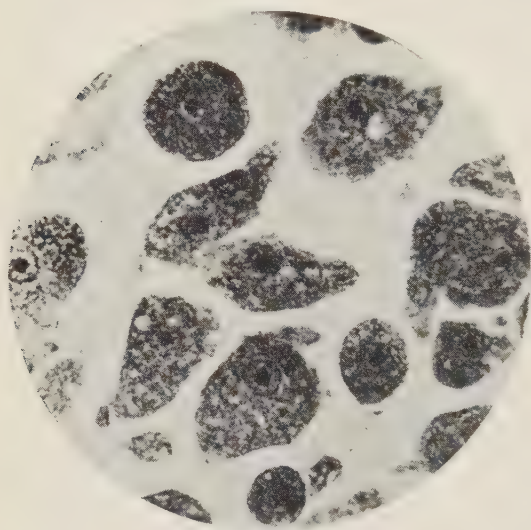


FIG. 23.—*Vahlkampfia lobospinosa*.  $\times 750$ . (Photomicrograph, Army Medical School Collection.) Stained with iron hæmatoxylin. Numerous vegetative forms of *Vahlkampfia lobospinosa*. Note character of the nucleus and the contractile vacuole in some of the organisms.

cysts are produced, usually quadrilateral or pyramidal in shape. The old cysts have a very thick wall, which may appear cracked or folded over in places, thus giving the cyst a very characteristic appearance. No bacteria are present in the cysts and the nucleus is often invisible in the living cyst, the cytoplasm appearing to be filled with minute refractile granules.

In stained cysts the cytoplasm appears granular and the nucleus presents the same structure as that observed in the vegetative stage. I have never observed

more than one nucleus in the cyst of this amœba, nor have I observed any evidence of the division of the nucleus in the cyst. When conditions are favorable one amœba emerges from the cyst in every instance, so far as I have been able to observe.

Williams and Calkins (1913) claim to have cultivated this species in pure culture upon brain-tissue medium and that upon this medium the contractile vacuole disappears, the nuclei resemble in structure the nuclei of the endamœbæ, and the cysts become multinucleate, from two to four nuclei being present in some of the cysts. Regarding the significance of these changes they say:

"We do not wish, however, to lay too much stress upon these experimental results; conditions of the culture might be such as to bring about variations when these do not occur under conditions 'normal' to the organism."

However, their observations are of great interest as showing how



profoundly a protozoan organism may be modified by unnatural cultural conditions, but there is no reason to believe that these modifications in any way connect this typical free-living amœba with the parasitic amœbæ of man, for even the modified nucleus in the amœbæ in Williams and Calkins cultures are very different in appearance from the nucleus of the parasitic amœbæ, and the cysts, even though multinucleated, do not resemble closely those of the parasitic amœbæ. The experiments of Walker and Sellards (1913) have settled definitely, in my opinion, the question of any possible relation of the free-living amœbæ to the parasitic species and their relation to disease. Their feeding experiments with cultures of free-living amœbæ demonstrated that these amœbæ are unable to live as parasites in man and that when such amœbæ are found in cultures from stools, liver-abscess pus, or intestinal contents, they are derived either from contamination of the cultures or from encysted free-living amœbæ which have been ingested in food or drink.

I believe that it is possible, however, for certain free-living amœbæ to excyst in the human intestine and to live for a very limited period in that organ. I have seen amœbæ in the stools, in a few instances, in the vegetative stage, that were indistinguishable morphologically from *Vahlkampfia lobospinosa*, and I do not believe that these amœbæ could have contaminated the stools after passage as the utmost care was taken to avoid such contamination. In every such instance the amœbæ disappeared within two or three days after being first noticed, but I am convinced that the vegetative forms really occurred in the fæces when voided.

*Vahlkampfia lobospinosa* can be very easily cultivated upon Musgrave and Clegg's agar medium and the cultures can be continued indefinitely. This amœba was carried along in cultures at the Army Medical School for a period of over twelve years, and could have been carried along indefinitely, without doubt. The cysts are very resistant to drying, and I have obtained cultures of the vegetative forms from cysts that had been dried on culture plates for several months, but in all the years during which this amœba was under observation there was never the least change in morphology or in its method of development or reproduction.

This species has been described in some detail because it is apparently frequently obtained in cultures from stools and has been confused with the parasitic amœbæ of man. The free-living amœba described by Hartmann and named by him *Vahlkampfia whitmorei* may be identical with this species.

There are other species of coprozoic amœbæ described as being found in human stools, but the species considered are the most important and most frequently encountered. In the differential diagnosis of these organisms the structure of the nucleus and the character of the cysts must be depended upon to separate them from the parasitic amœbæ

described, but the most useful differential feature is the fact that they can all be easily cultivated upon the simple agar medium of Musgrave and Clegg, and that none of the parasitic amœbæ can be so cultivated.

### BLASTOCYSTIS HOMINIS Brumpt, 1912

This vegetable organism is often confused with cysts of parasitic amœbæ or flagellates. Prowazek (1904) described it as the cystic stage of *Trichomonas hominis*. The organism occurs in the stools of man in the form of small spherical bodies surrounded by a narrow layer of cytoplasm which contains nuclei. The same bodies were later described by Benson (1910), and Wenyon, in 1910, considered them as the cystic stage of *Chilomastix mesnili*. In 1911, Alexeieff studied these organisms and concluded that they were not protozoan in nature, but belonged to the BLASTOMYCETES. He considered that they constituted a new species and named the organism *Blastocystis enterocola*. Other authorities, as Brumpt (1912), Mathis (1913), and Low (1916), confirmed the findings of Alexeieff, and Brumpt (1922) proposed the name *Blastocystis hominis* for the species found in man. Swellengrebel (1917) regards these organisms as being degeneration forms of certain intestinal protozoa, but this opinion is negated by the recent studies of Lynch (1922), who succeeded in cultivating these organisms and described three distinct species, i.e., *Blastocystis hominis*, Brumpt, 1912; *Blastocystis gemmagina*, Lynch, 1922; and *Blastocystis spirogina*, Lynch, 1922. The latter species he regards as uncertain. According to Lynch these species differ in their morphology and methods of reproduction, but until his work has been confirmed I believe that it is best to regard all organisms of this class occurring in the human intestine as belonging to one species, i.e., *Blastocystis hominis*.

*Blastocystis hominis* in the living condition appears as a colorless, refractile, hyaline, spherical body, measuring from 5 to 30 microns or more in diameter, the average size being from 10 to 15 microns in diameter. Most of the organism consists of a circular mass, hyaline in appearance, and showing no definite structure, which is surrounded by a narrow band of cytoplasm containing refractile granules and one or more larger, refractile, round or oval masses, the nuclei. The entire organism is surrounded by a delicate membrane and in some specimens this is again surrounded by a definite capsule. In stained specimens the narrow band of cytoplasm contains well-stained nuclei which appear as very minute bodies, round or oval in shape, and having a relatively large karyosome which stains intensely with hæmatoxylin. A few minute grains of chromatin may also be present in this portion of the cytoplasm. Dividing forms are often observed, a constriction being present at or near

the centre of the organism, thus producing an hour-glass appearance. In cultures, and also in the fæces, considerable variation occurs in the morphology of the organisms, probably because of mixed infections with the different species. See Figs. 24, 25.

*Blastocystis hominis* occurs in the fæces of almost every one, and for this reason its recognition is important, as it has been so often confused, even by trained protozoologists, with protozoan organisms occurring in the human intestine. If one remembers its peculiar structure it can be easily differentiated from any of the cysts of parasitic protozoa occurring in man, and it is so commonly present, material for its study is unlimited and no excuse exists at the present time for such confusion.

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## CHAPTER V

### THE DIAGNOSIS OF THE PARASITIC AMŒBÆ OF MAN

THE diagnosis of infection with parasitic amœbæ can only be made by the finding of the amœbæ in the stools, or, in the case of *Endamœba gingivalis*, in the mouth, of the individual suspected of harboring these parasites. Diagnosis cannot be made from the clinical symptoms present, for most of the parasitic amœbæ are non-pathogenic, and even in the case of *Endamœba histolytica*, the cause of amœbic dysentery, clinical symptoms are often absent and, when present, are often atypical. An accurate diagnosis can be made only by a microscopical examination and the actual demonstration of the amœbæ in the material examined.

**The Diagnosis of the Parasitic Amœbæ of the Human Intestine.**—Prior to the publication of Schaudinn's paper, in which he clearly differentiated *Endamœba histolytica*, the pathogenic amœba of man, and *Endamœba coli*, the common, harmless amœba of the human intestine, the finding of amœbæ in the stools of an individual was considered as diagnostic of amœbic dysentery, and multitudes of individuals were treated for such infection, even in the absence of symptoms, when they were really infected with the harmless *Endamœba coli*. With our present knowledge of the existence of at least five different species of amœbæ living in the intestine of man, it is not only necessary to diagnose the presence of an amœba in a suspected case, but also, and much more important, to make a differential diagnosis of the exact species present, before one is justified in concluding that diarrrhœal or dysenteric symptoms that may be present are due to amœbæ and that the case is one of amœbic dysentery.

The data which were collected prior to Schaudinn's work, and much of that which has been published since, purporting to show the prevalence of amœbic dysentery in different localities, are worthless, because the species of amœbæ present in the individuals examined were not differentiated, and it is only within recent years that reliable statistics, based upon a differentiation of the species of amœbæ present in the stools, have been collected and published.

No diagnosis of amœbic dysentery is of any scientific value unless *Endamœba histolytica* is demonstrated in the stools of the patient, and it is necessary in diagnosis to differentiate this species from *Endamœba coli*, *Endamœba nana*, *Iodamœba williamsi*, and *Dientamœba fragilis*. In order to do this it is essential that one have a knowledge of the morphology of the parasitic amœbæ of the human intestine and of other protozoa occurring in this region, as well as of various vegetable cells

that occur in the stools, a knowledge that cannot be acquired by the cursory examination of a few specimens of fæces, but is always the result of many months of careful study of the protozoa of the intestine and the experience gained by many errors in the diagnosis of the various organisms that have been encountered.

Fortunately, the differential diagnosis of *Endamæba histolytica* can be made in acute cases of amœbic dysentery upon comparatively simple criteria, so that in this class of cases the differential diagnosis of this species is not so difficult, but in subacute and chronic infections, where the diagnosis must rest upon the character of the pre-cystic and cystic stages of the amœbæ, diagnosis is more difficult, and should not be attempted by one who has had little, or no, experience in the examination of stools for protozoa.

It is true that, in the vast majority of cases, a differential diagnosis need not be made between all of the intestinal amœbæ and *Endamæba histolytica*, but only between this parasite and *Endamæba coli* and *Endamæba nana*, as the other intestinal amœbæ, *Iodamæba williamsi* and *Dientamæba fragilis*, are less frequently encountered, especially the latter. However, the importance of making a differential diagnosis between these parasites is apparent if our statistics regarding the prevalence of amœbic dysentery are ever to approach scientific accuracy, and if our efforts at prophylaxis and treatment of this serious infection are to be efficient. The mere finding of an amœba in the stools means nothing unless the species be identified, and at the present time the diagnosis of amœbic dysentery based on anything less than the identification of the amœba present as *Endamæba histolytica* is unjustifiable and valueless.

The differential diagnosis of *Endamæba histolytica*, *Endamæba coli*, and *Endamæba nana* rests upon the morphological characters of these parasites as observed in living specimens and in stained preparations, during the vegetative and cystic stages of development. In the vast majority of instances a differential diagnosis may be made between them by studying fresh, unstained material containing them and material stained with the iodine solution. In doubtful cases it is necessary that staining be employed, and for this purpose one of the hæmatoxylin stains described in the Appendix should be used. For diagnostic purposes it is not generally necessary to resort to staining with the hæmatoxylin stains, but for the study of the finer structure of the nucleus such stains are necessary.

**Technique of Examinations. Examination of Living Specimens.**—In selecting material for examination from the stools for amœbæ the most important point to remember is that the fresher the material is, the better. These organisms degenerate very quickly after the passage of the stool containing them unless they are in the cystic stage of develop-

ment, so that if it is desired to study the living, motile organisms, or to secure stained preparations showing the typical structure of the nucleus, it is absolutely essential that the preparations be made from a freshly passed stool. The stool should be collected in a clean receptacle which does not contain water or any antiseptic and should be sent to the laboratory at once for examination.

With the exception of *Dientamœba fragilis*, which is a very rare species, all of the intestinal amœbæ of man may be found in the motile stage in stools that have been passed, sometimes, for several hours, but such amœbæ are not suitable for study, as amœboid motility is either absent or very sluggish, and the structure of the nucleus in these amœbæ generally shows some abnormality, thus interfering with a differential diagnosis. The safest rule to follow in the examination of these parasites is to undertake a differential diagnosis only when freshly passed stools are available.

Each of the parasitic amœbæ of the human intestine has three well-marked stages in its life-history: a vegetative, or motile, stage; a pre-cystic stage; and a cystic stage. The vegetative stage occurs generally only in fluid or semi-fluid stools, the pre-cystic stage in semi-fluid and formed stools, and the cystic stage in formed stools. Sometimes any of the stages may be encountered in the bloody, mucoid stools of infections with *Endamœba histolytica*, but it is very rare to find cysts of this species in such stools unless the infection be a chronic one, during an acute exacerbation. These facts should be remembered in examining material, and if it is desired to study the motile amœbæ, and the stools are formed, a saline purgative should be given, which will often cause the motile forms to appear in the stools, while if it is desired to study the cysts, a purgative should not be administered, as these forms of the amœbæ occur in the semi-formed or formed stools.

If the fæces are fluid, as in acute cases of amœbic dysentery, and contain mucus and blood, a small amount of bloody mucus is taken up with a platinum loop, placed upon a microscopic slide, covered with a cover-glass, and examined at once with a one-sixth- or one-eighth inch dry objective and a low ocular, using a mechanical stage upon the microscope for convenience of examination. A warm stage is not necessary unless it is desired to follow the movements of the amœbæ for some time, when it will be found essential.

If the fæces are semi-formed or formed, a small portion is emulsified with normal saline solution, placed upon a microscopic slide, covered with a cover-glass, and examined. Care should be taken that the specimen is not made too thick to see through, and only experience will teach one just how much material should be used in order to secure a satisfactory preparation.

If the stools are formed it will often be found that mucus or blood is present externally, and if so, the specimen should be taken from such material. This often occurs in old chronic amœbic infections, the hard fæcal mass scraping blood and mucus from the partially healed ulcerations during its passage to the rectum. Such material will often contain motile *Endamæba histolytica*, but unless a careful inspection is made of the stool, such material is generally overlooked, and the diagnosis may be missed.

In examining fresh living specimens, the iris diaphragm of the microscopic condenser should be closed as much as possible, for if it is widely open the amœbæ will not be seen, owing to their transparency and lack of color. The oil immersion objective is not necessary in the diagnosis of intestinal amœbæ, but is essential in the study of the structure of the nucleus in hæmatoxylin-stained specimens.

While it is necessary that the fæces be examined as quickly after passage as possible if one desires to study the living, motile vegetative forms of the intestinal amœbæ, it is not necessary that absolutely fresh material be examined if the cysts are being sought, as fæces even a day or two old will show cysts that can be differentiated. However, it is always best to examine the stools as soon after passage as possible, even if they are formed and cysts only are present, and if stained preparations are to be made the stool should always be as freshly passed as possible.

**Stained Specimens.**—With the exception of the use of the iodine solution in staining the cysts of the intestinal amœbæ, staining is not necessary, in the vast majority of instances, for diagnostic purposes, but the use of the iodine solution in the differentiation of the cysts is a most valuable clinical method and one that should always be employed in the examination of the cysts.

**Iodine Stain for Cysts.**—The solution employed for staining with iodine is a one per cent. watery solution of potassium iodide saturated with iodine.

A small portion of the fæces to be examined is emulsified with the iodine solution upon a microscopic slide and covered with a cover-glass. The preparation should be allowed to stand for at least ten minutes before being examined, being ringed with vaseline to prevent evaporation. At the end of this time the preparation may be examined with the one-sixth or one-eighth dry lens, and the cysts will appear as slightly yellowish or brown bodies within which the nuclei are distinctly visible and can be easily counted, and the morphological details noted. If glycogen vacuoles are present they will appear as mahogany-brown areas within the cysts. With this stain it is perfectly possible to differentiate the cysts of the various species of amœbæ occurring in the intestine of man. The



stain is of no service in examining the motile vegetative stage of the amœbæ.

**Permanent Stained Preparations.**—When it is desired to study the finer details of structure of the amœbæ, or when it has been impossible to make a differential diagnosis of the species of amœba present, the use of permanent stained specimens is necessary, and many different methods have been devised for fixing and staining the intestinal amœbæ. In order to secure good specimens it is absolutely necessary that the material be secured from a freshly passed stool, and before staining the material must be fixed while still moist. The fixing solution usually used consists of a saturated solution of bichloride of mercury in water, two parts; absolute alcohol, or 96 per cent. alcohol, 1 part. Mix and add to each 100 c.c. of the mixture, 5 c.c. of glacial acetic acid. This solution will keep for months.

A small portion of bloody mucus, or an emulsion of the fæces, if it be formed, is smeared with a platinum loop upon a cover-glass and immediately placed film side downward upon the fixing solution contained in a small Petrie dish or watch-glass, or the cover-glasses may be allowed to sink in the solution film side upward, if preferred. Fixation is accomplished in from 10 to 20 minutes, the vegetative amœbæ being fixed in from 10 to 15 minutes, but the cysts should be allowed the full time.

After fixation the film should be thoroughly washed by being carried through 50 per cent. alcohol, 70 per cent. alcohol, and 70 per cent. alcohol to which a few drops of the iodine solution already described has been added, enough to give the mixture a port-wine color. In this the films should remain for 30 minutes and then be placed in 35 per cent. alcohol for a few minutes, after which they are transferred to water. After remaining in the water for a few minutes the films are stained for from 10 to 20 minutes with the following staining solution: Hæmatoxylin, 1.0 gm.; distilled water, 1,000 c.c. Dissolve the hæmatoxylin and then add: Sodium iodate, 0.2 gm., potash alum, 50 gms. Mix and filter. This staining solution is known as Mayer's "Hæmalum," and should be red in color when properly prepared. It should be freshly made each time that it is used in order to secure the best results.

The vegetative amœbæ are more easily stained than the cysts, and the latter should be allowed to remain in the solution for the full 20 minutes, and sometimes longer. If the specimens are overstained they may be decolorized with acid alcohol.

After staining, the preparations are washed carefully in tap water until they assume a bluish color, and are then dehydrated and mounted. Dehydration is accomplished by carefully carrying the preparations through 35 per cent., 50 per cent., 70 per cent., and 90 per cent. alcohol,

and finally into absolute alcohol, where they should remain for at least five minutes. From the absolute alcohol the specimens are transferred to a mixture of equal parts of absolute alcohol and xylol, and allowed to remain for five minutes, after which they are cleared in pure xylol, and mounted in xylol balsam.

It is quite difficult to obtain really excellent stained specimens until one has had much experience, and *it is absolutely necessary to be sure that at no stage of the fixing, staining, or dehydration process do the preparations become dry*. They must be kept wet throughout the entire process or poor results will be obtained. It is also necessary that the dehydration process be carefully conducted and at least from 5 to 10 minutes be allowed in each solution before advancing the preparation to the next solution.

With this stain the cytoplasm of the amœbæ appears of a bluish-gray color, while the nucleus is well defined, the nuclear membrane, the karyosome, and the chromidial bodies staining a deep brown or black.

Many other methods of fixing and staining the amœbæ have been recommended by various observers, and those that have been found most valuable will be found described in the Appendix. The method given above is the one I have found to be reliable in diagnosis, and as simple as any that have been described. It does not give as beautiful preparations as the Rosenbusch or Mann method (See Appendix), but it is satisfactory in clinical diagnosis.

**The Differential Diagnosis of the Vegetative Forms of *Endamœba histolytica*, *Endamœba coli*, and *Endamœba nana*.**—*Unstained Preparations*.—As these are the three most common intestinal amœbæ of man, and as it is necessary to differentiate *Endamœba histolytica* from *Endamœba coli* and *Endamœba nana* most frequently, the differential diagnosis of these species will first be considered.

The morphology of these parasites in the vegetative, or motile, stage varies considerably, but in freshly passed stools the three species may be differentiated without much difficulty. If old stools are examined the organisms will be found to vary exceedingly in morphology due to the degenerative changes that occur, and many of the mistakes that have been made in the description of these amœbæ, especially as regards their nuclear structure, were due to the study of stained preparations of degenerated amœbæ.

*Size*. With the exception of *Endamœba nana*, size is of little importance in the differentiation of these amœbæ. While *Endamœba histolytica*, as observed in the stools in acute amœbic dysentery, is usually larger than *Endamœba coli*, individual specimens of *coli* are observed which are as large, or larger, than the average *histolytica*, so that a differentiation of these two species based alone upon size is impossible.

However, in the case of *Endamæba nana*, the fact that it is smaller than even the average of *Endamæba coli* or *Endamæba histolytica* renders the size of this parasite of some diagnostic importance. The average diameter of *Endamæba histolytica*, in the vegetative stage, varies from 20 to 35 microns; that of *Endamæba coli* from 20 to 30 microns; while that of *Endamæba nana* varies from 6 to 12 microns, the average diameter being about 8 microns, a little larger than the normal red blood corpuscle.

*Motility.* In the freshly voided stools the vegetative stage of *Endamæba histolytica* is very actively motile, and motility is of a markedly progressive character, the parasite moving forward by means of rapidly extruded pseudopodia formed of the ectoplasm. In *Endamæba coli* the motility is very sluggish and rarely progressive in character. When progressive motion is observed it is very slow and creeping rather than rapidly rolling as in *histolytica*. In *Endamæba nana*, unless the specimens be examined immediately after passage of the stool, no motility is observed. When present it is very sluggish and there is seldom any progressive motility, the pseudopodia being extruded and withdrawn with practically no progressive motion ensuing. If an amœba is observed in the stools which shows rapid progressive motility it is, in all probability, an example of *Endamæba histolytica*, for in my experience neither *Endamæba coli* nor *Endamæba nana* ever show such rapid progressive motility.

*The Pseudopodia.* The pseudopodia are formed of the ectoplasm in all three species and their character is of diagnostic value. In *Endamæba histolytica* the pseudopodia are generally large, finger- or blade-shaped, and perfectly clear and glass-like in appearance. They give the impression of being of considerable consistency and strength. The pseudopodia of *Endamæba coli* are much smaller, rather broad and blunt, and do not present the glass-like appearance of those of *histolytica*. They appear to be more fragile, and are never long and finger- or blade-shaped as in *histolytica*. The pseudopodia in *Endamæba nana* are short and blunt, very minute in size in comparison with either *histolytica* or *coli*, and have a very weak and fragile appearance. The pseudopodia are so characteristic in the case of *Endamæba histolytica* that one may almost make a diagnosis of the species from this morphological feature alone, and when an amœba is observed in the stools showing such pseudopodia, accompanied by rapid progressive motion, the diagnosis may be made without hesitation.

*Ingested Material.* The endoplasm of *Endamæba histolytica* is free from vacuoles and bacteria in specimens which are present in freshly voided fæces or in amœbæ removed from ulcers in the intestine. After the fæces have stood for some time the endoplasm of this species may



show numerous vacuoles and many bacteria, but the appearance of these always indicates degeneration of the amœbæ. Food vacuoles may be present and may contain leucocytes, tissue cells, or red blood corpuscles. *The ingestion of red blood corpuscles by this species is a most important diagnostic character, for these cells are seldom, if ever, ingested by either Endamæba coli or Endamæba nana, and the presence of an amœba in a stool which contains within its cytoplasm red blood corpuscles is absolutely diagnostic of this species, in the opinion of all students of the subject.* I have always urged the great value of the phagocytosis of red blood corpuscles in the differentiation of *Endamæba histolytica*, and my observations in this respect have been confirmed by Wenyon and O'Connor and Dobell, who state that an amœba showing red blood corpuscles within its endoplasm may safely be diagnosed as *Endamæba histolytica*. The ingestion of red blood corpuscles by *Endamæba coli* has been described by myself and others, but in the light of accumulated experience it is probable that these organisms were really specimens of *Endamæba histolytica* and were mistaken for *Endamæba coli*, there being a mixed infection with the two species present.

The endoplasm of *Endamæba coli* is always filled with food vacuoles in which bacteria, starch grains, intestinal débris, and even the cysts of intestinal flagellates may be found. The vacuole-filled cytoplasm of *Endamæba coli* is in marked contrast to the homogeneous-appearing cytoplasm of *Endamæba histolytica*, for the latter species does not contain numerous vacuoles unless degeneration is occurring.

The endoplasm of *Endamæba nana* is also filled with food vacuoles containing bacteria, but red blood corpuscles are never present in the cytoplasm of this species.

*The Nucleus.* The nucleus of *Endamæba histolytica* is not visible in the vegetative stage of growth in the living specimen unless the fæces containing this parasite have stood for some time at room temperature, when it may become visible as a ring of refractile granules. The nucleus of *Endamæba coli* is generally visible in the living vegetative organism as a ring of rather large refractile granules containing within it a refractile mass representing the karyosome. The nucleus of *Endamæba nana* is seldom visible in the vegetative stage in the living amœba.

*Summary.* In the living vegetative forms of the three amœbæ under consideration, a differential diagnosis is possible if attention is paid to the differences in morphology that have been described and that are summarized in the diagnostic table which follows. Briefly summarized, it may be stated that if one finds in the stool under examination a large, actively moving amœba, showing marked progressive motion, the pseudopodia being well differentiated from the endoplasm, and finger- or blade-shaped, while the endoplasm contains red blood corpuscles and is free



from numerous vacuoles and bacteria, one would be justified in making a diagnosis of *Endamæba histolytica*. On the other hand, if the amœbæ present were only sluggishly motile, the pseudopodia poorly differentiated, and no progressive motion is present, while a distinct nucleus is present, and the endoplasm does not contain red blood corpuscles even though blood may be present in the stools, and the cytoplasm is filled with vacuoles and bacteria, the diagnosis would be *Endamæba coli* or *Endamæba nana*, the latter being differentiated by its small size from either *Endamæba coli* or *Endamæba histolytica*.

**Vegetative Stage.**—*Stained Preparations.* In stained preparations the differential diagnosis between the three species under consideration rests largely, aside from the size of the organisms and the presence or absence of red blood corpuscles in the cytoplasm, upon the character of the nucleus. The structure of the nucleus in these amœbæ has been fully considered in the description of the individual organisms and is summarized in the diagnostic table which follows. It may be stated that a careful consideration of the differences in the structure of the nucleus of these three species of amœbæ should be sufficient to enable one having experience in the study of the intestinal protozoa to easily differentiate these species, but it should be remembered that, in order to make such a differentiation, it is absolutely essential that the preparations be made from freshly voided stools, as the nucleus of all of these organisms undergoes marked degeneration within a short period after the passage of the stool.

In addition to the character of the nucleus in the species of amœbæ under discussion, some help in differentiation in stained specimens is given by the character of the cytoplasm. In *Endamæba histolytica* the cytoplasm appears granular and homogeneous and is free from vacuoles unless degeneration is occurring, and bacteria and other ingested material, with the exception of red blood corpuscles or tissue cells and leucocytes, are not present. If red blood corpuscles are present the diagnosis of *Endamæba histolytica* is justified. The cytoplasm of *Endamæba coli* is filled with vacuoles, and bacteria, crystals, and other ingested material are commonly present, while the cytoplasm of *Endamæba nana* is also much vacuolated, and bacteria are usually present. Red blood corpuscles do not occur in the cytoplasm of either *Endamæba coli* or *Endamæba nana*.

**The Differential Diagnosis of *Endamæba histolytica*, *Endamæba coli*, and *Endamæba nana* in the Pre-cystic Stage of Development.**—While in certain instances it is possible to differentiate between *Endamæba histolytica* and *Endamæba coli* in the pre-cystic stage of development, in the vast majority of specimens that one examines it is impossible to do so, in either fresh or stained preparations, and for this reason the diagnosis

should be based upon the character of either the vegetative or cystic forms that are fortunately present in smaller number in material containing the pre-cystic forms, in most instances. If only pre-cystic forms of these two amœbæ be present, one should be very cautious in giving an opinion as to which species is present, and it is best to never make a diagnosis upon these forms alone. The pre-cystic forms of *Endamœba nana* are likewise very hard to distinguish from either *histolytica* or *coli* if the forms of the latter species are those that will produce the small races of cysts, but in the pre-cystic forms of *Endamœba nana* the character of the nucleus is retained and serves to distinguish the pre-cystic form of this species.

**The Differential Diagnosis of the Cysts of *Endamœba histolytica*, *Endamœba coli*, and *Endamœba nana*.**—*Unstained Preparations.* In the living unstained condition the cysts of all three species appear in the preparations as colorless, refractile, hyaline bodies. The cysts of *Endamœba histolytica* and *Endamœba coli* are usually circular in shape, although they may be oval or irregular, while those of *Endamœba nana* are generally oval, but may be circular or irregular. There is much greater variation in the shape of the cysts of *nana* than in the cysts of either *histolytica* or *coli*.

*Size.* The cysts of *Endamœba histolytica* and *Endamœba coli* vary greatly in size, as there are several races of both parasites characterized by the production of cysts of different size. Thus, some races may produce cysts as small as 5 microns in diameter, while others produce cysts measuring over 20 microns in diameter. As a rule, the cysts of *Endamœba coli* are larger than those of *Endamœba histolytica*, while the cysts of *Endamœba nana* are usually smaller than either those of *histolytica* or *coli*.

In the unstained specimen there is little that distinguishes the cysts of these three species from one another beyond the difference in size and, in the case of *Endamœba nana*, the oval shape of the cyst. The nuclei can seldom be distinguished or counted in the unstained specimens, and for this reason such preparations are of little value in diagnosis.

*Stained Preparations.* In preparations stained with the iodine solution, which is especially useful in routine diagnostic work, it is possible to differentiate the cysts of the three species under discussion with little difficulty, in most instances, by attention to the number of nuclei present. The iodine solution renders the nuclei visible and it is possible to count them. The cysts of *Endamœba histolytica* contain from 1 to 4 nuclei, the fully developed cyst containing 4 nuclei; the cysts of *Endamœba coli* contain from 1 to 8 or more nuclei, the normal, fully developed cyst containing 8 nuclei; while the cysts of *Endamœba nana* contain from 1 to 4, or rarely, 8 nuclei, the normal, fully developed cyst containing 4

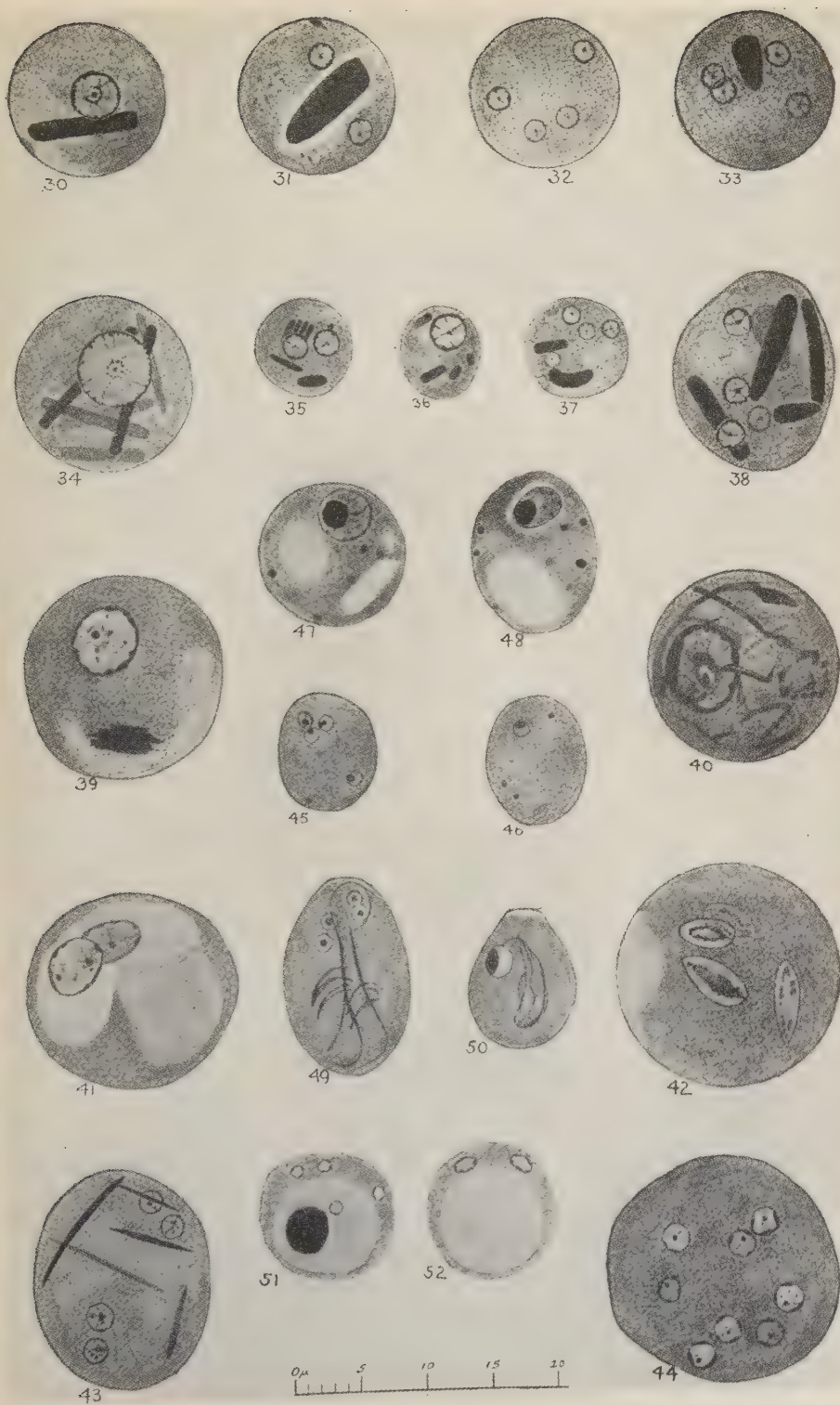


FIG. 24.—See explanation on next page.



nuclei. The cysts of *Endamæba coli* may contain as many as 24 nuclei, and cysts containing as many as 16 nuclei are not so very uncommon, but I have never seen more than 4 nuclei in the cysts of *Endamæba histolytica*. The 4-nucleated cyst of *Endamæba nana* is distinguished from that of *Endamæba histolytica*, in iodine preparations, by its oval shape and generally smaller size.

In preparations wet-fixed and stained with one of the hæmatoxylin stains, the differential characteristics of the various cysts are more easily distinguished and consist of the number and structure of the nuclei present, and the morphology of the chromidial bodies within the cysts.

The number of nuclei characteristic of each species in the cystic stage of development has been stated, and the morphology of these nuclei is similar to that of the typical nucleus of each species as observed in the vegetative stage of development. The nuclei of the cysts of *Endamæba histolytica* present the delicate nuclear membrane, the minute, centrally situated karyosome, and the absence of chromatin between the

#### EXPLANATION OF FIGURE 24

Protozoan cysts as they appear when stained with iron hæmatoxylin. (After Boeck. Bull. 133. Hyg. Lab. U. S. Public Health Service.) Explanation of figures quoted from Boeck.  $\times 1,800$ .

*Endamæba histolytica*.—Figures 30, 31, 32, 33 (medium size race); 34, 38 (large size race); 35, 36 and 37 (small size race). Cytoplasm, finely granulated, may show empty vacuole (fig. 30) which previously contained a glycogen mass. Chromatoidal bodies with rounded ends stain black, more numerous in uninucleate cysts (fig. 34). Nuclei show small beads of peripheral chromatin, on fine nuclear membrane, a minute central chromatin granule, the karyosome, and radiating achromatic fibres. Nuclei may be 1, 2 and 4, rarely 8 in number; 4 nucleated cysts most prevalent in a stool.

*Endamæba coli*.—Figures 39, 40, 41, 42, 43 and 44. Cytoplasm more coarsely granulated than that in *E. histolytica* cysts. Vacuoles usually present in 1 and 2 nucleated cysts (figs. 39, 41, 42). Chromatoidal bodies, with pointed ends, or like ribbons, may occur in 1, 2 and 4 nucleated cysts (figs. 39, 40, 43), seldom in 8 nucleated cysts. Nuclei show large beads of peripheral chromatin on fairly thick nuclear membrane and a large and eccentric karyosome compared with the minute central karyosome in *E. histolytica* cysts. Radiating achromatic fibres often visible in nuclei; they often suspend granules of peripheral chromatin (figs. 39, 41, 42, 43). Dividing nuclei, spindle shaped (fig. 42), the chromatin granules suspended upon spindle fibres.

*Endamæba nana*.—Figures 45 and 46. Cytoplasm uniformly granulated may contain a vacuole (previously occupied by glycogen) and round, black granular inclusions. Nuclei, 1 to 4 in number, small, each with an indistinct nuclear membrane upon which lies a large karyosome; the latter may be joined by a fine fibre to one or more additional chromatin masses (fig. 45).

*Iodamæba williamsi*.—Figures 47 and 48. Cytoplasm contains numerous black granules and 1 or more empty vacuoles. Single large nucleus, somewhat indistinct nuclear membrane, large eccentric karyosome, meshwork of achromatic fibres suspending granules of peripheral chromatin.

*Giardia lamblia*.—Figure 49. Cytoplasm uniformly granulated, contains 2 long black strands (axostyles) and 1 or 2 pairs of black curved bodies (parabasals), and remains of right anterolateral flagellum. Four nuclei, with distinct membranes and large central karyosomes, present.

*Chilomastix mesnili*.—Figure 50. Cytoplasm finely granulated; contains cytostomal fibres arising from blepharoplast complex. Nucleus with distinct nuclear membrane, large eccentric karyosome. Centrosome at anterior pole of nucleus connected to blepharoplast complex by fine fibre or rhizoplast.

*Blastocystis hominis*.—Figures 51 and 52. Vegetable organisms with 4 and 2 nuclei, respectively, and with a central vacuole either empty or containing a black staining inclusion. Cytoplasm limited to a thin wall surrounding the vacuole.



karyosome and the nuclear membrane, as in the vegetative forms; the nuclei of cysts of *Endamæba coli* show the thicker nuclear membrane, the larger, eccentrically situated karyosome, and the granules of chromatin between it and the nuclear membrane, as in the vegetative forms; while the nuclei of the cysts of *Endamæba nana* present the same peculiar divided karyosome which has been described as characteristic of this amœbæ in the vegetative stage of development.

*Chromidial Bodies.* Chromidial bodies occur in a certain proportion of the cysts of *Endamæba histolytica* and *Endamæba coli*, and are of great diagnostic importance. These bodies stain black with the hæmatoxylin stains and are easily distinguished. The iodine stain renders them distinct enough to study, and with both stains they appear in *Endamæba histolytica* as large bar-like, oval, or spindle-shaped masses with rounded ends lying in the cytoplasm of the cyst, the nuclei lying between or around them. In *Endamæba coli* the chromidial bodies are quite different in character, the cysts containing long, slender acicular bodies with fractured or sharp ends, resembling a bundle of slender crystals, in many instances. The large blocks or bars of chromatin with rounded ends are never observed in the cysts of this species. *Endamæba nana* cysts do not show any chromidial bodies comparable to those noted in *Endamæba histolytica* or *Endamæba coli*, but small granules and rods of some material staining like chromatin occur in the cysts, the nature of which is uncertain. The following table gives the most important differential diagnostic features between *Endamæba histolytica*, *Endamæba coli*, and *Endamæba nana*, the three most common parasitic amœbæ of the human intestine.

DIAGNOSTIC FEATURES IN THE DIFFERENTIATION OF *ENDAMÆBA HISTOLYTICA*, *ENDAMÆBA COLI*, AND *ENDAMÆBA NANA*

	<i>Vegetative Stage of Development. Living Specimens.</i>		
	<i>Endamæba histolytica</i>	<i>Endamæba coli</i>	<i>Endamæba nana</i>
Size.	18 to 80 microns. Average, 20 to 35 microns.	15 to 50 microns. Average, 20 to 30 microns.	6 to 12 microns. Average, 8 microns.
Motility.	Very active and progressive.	Sluggish. Rarely progressive.	Sluggish. Not progressive.
Cytoplasm.	Ectoplasm and endoplasm well differentiated in motile organisms.	Ectoplasm and endoplasm poorly differentiated.	Ectoplasm and endoplasm poorly differentiated.
Pseudopodia.	Large, finger-shaped, clear and glass-like in appearance.	Shorter and blunt. Not glass-like in appearance.	Broad and blunt. Not glass-like in appearance.
Vacuoles.	Not present in normal living amœbæ.	Numerous vacuoles.	Numerous vacuoles.
Inclusions.	Red blood corpuscles. No bacteria or crystals.	Numerous bacteria, crystals, and other material. No red blood corpuscles.	Numerous bacteria. No red blood corpuscles.
Nucleus.	Generally invisible.	Visible.	Generally invisible.

DIAGNOSTIC FEATURES IN THE DIFFERENTIATION OF *ENDAMÆBA HISTOLYTICA*, *ENDAMÆBA COLI*, AND *ENDAMÆBA NANA*

*Vegetative Stage of Development. Stained Specimens.*

	<i>Endamæba histolytica</i>	<i>Endamæba coli</i>	<i>Endamæba nana</i>
Nuclear membrane.	Delicate. Inner surface lined with single layer of minute chromatin grains.	Thicker. Inner surface lined with coarser chromatin grains.	Intermediate in thickness. Chromatin grains rarely seen on inner surface.
Karyosome.	Minute. Situated in centre of nucleus.	About twice as large as in <i>E. histolytica</i> . Situated eccentrically.	Large and usually divided into one large and one or more small portions, connected by a delicate thread.
Intranuclear chromatin.	No chromatin between karyosome and nuclear membrane.	Chromatin grains between karyosome and nuclear membrane.	No chromatin between karyosome and nuclear membrane.
Cytoplasm.	Not vacuolated.	Much vacuolated. No red blood corpuscles.	Many vacuoles.
Inclusions.	Red blood corpuscles. No bacteria or crystals.	Bacteria, crystals, and other material.	Many bacteria. No red blood corpuscles.

*Cystic Stage of Development. Unstained Specimens.*

Size.	6 to 20 microns. Average, 7 to 15 microns.	10 to 22 microns. Average, 12 to 18 microns.	8 to 12 microns long by 7 to 10 microns broad.
Shape.	Generally spherical. Rarely oval or irregular.	Spherical. Rarely oval or irregular.	Oval or ellipsoidal. Sometimes spherical.

*Cystic Stage of Development. Stained Specimens.*

Nuclei, number.	1 to 4.	1 to 8. Sometimes more, up to 24.	1 to 4.
Nuclei, structure.	Like vegetative form but smaller, delicate membrane, minute karyosome centrally located, no chromatin between karyosome and nuclear membrane.	Like vegetative form but smaller. Thicker membrane, larger karyosome eccentrically situated and chromatin grains between karyosome and membrane.	Like vegetative form but smaller. Thick membrane, large, divided karyosome and no chromatin between membrane and karyosome.
Chromidial bodies.	Bar, oval, or rod-like masses with rounded ends. Present in about 50 per cent. of cysts.	Filamentous or acicular bodies with fractured or pointed ends. In less than 10 per cent. of the cysts.	None present comparable with those in <i>histolytica</i> or <i>coli</i> . Small granular or rod-like bodies are rarely observed.

**Differential Diagnosis of *Iodamœba williamsi* and *Dientamœba fragilis*.**—These amœbæ can be easily differentiated from the other parasitic amœbæ of the human intestine by attention to the peculiar structure of the cyst in *Iodamœba williamsi* and of the nucleus, and to the presence of two nuclei in *Dientamœba fragilis* and the absence of any cystic stage so far as has been determined. The "iodine cysts" of *Iodamœba wil-*

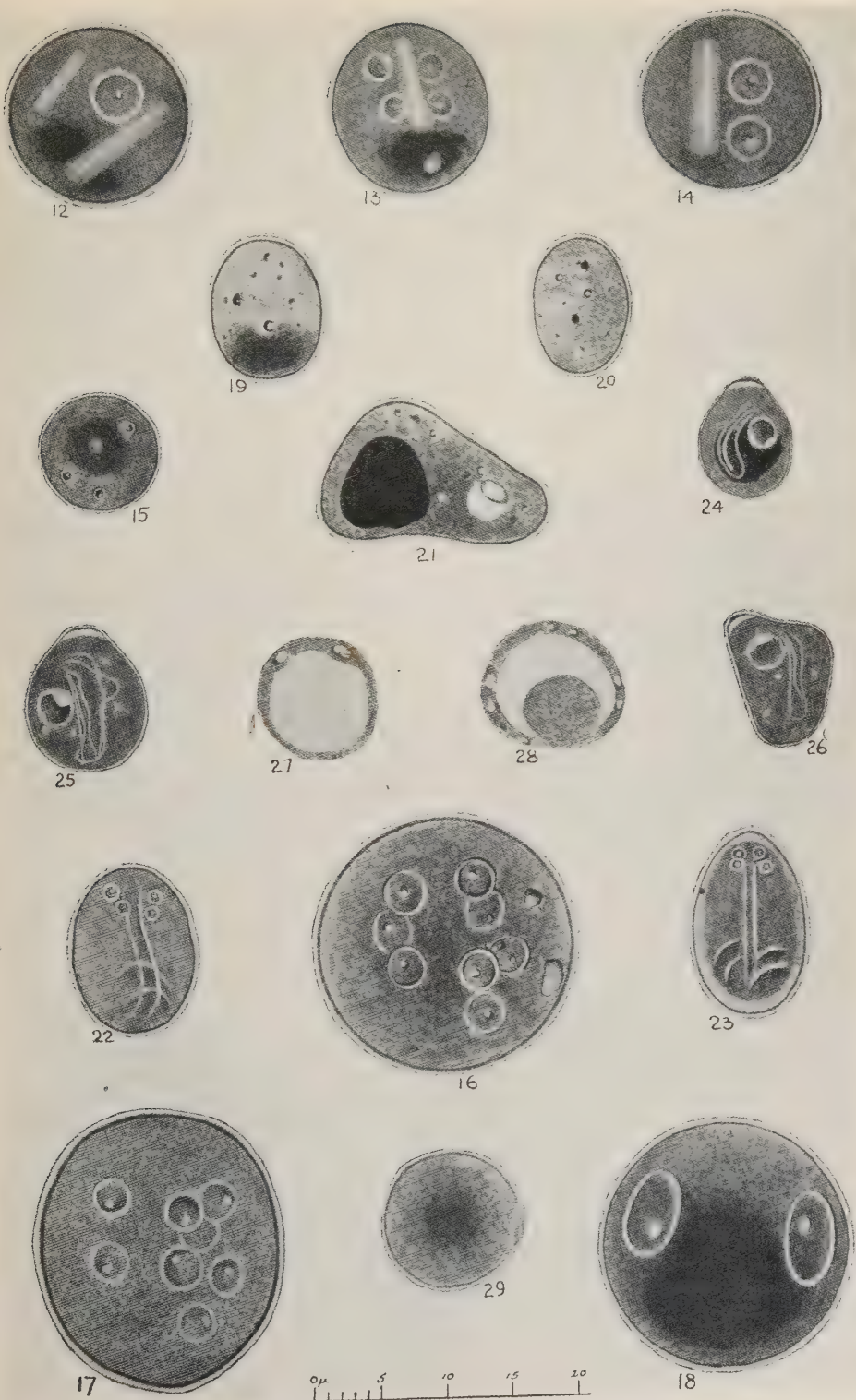


FIG. 25.—See explanation on next page.



*liamsi* are most characteristic and easily differentiated from the cysts of any other species of amœba occurring in the intestine.

**Coprozoic Amœbæ.**—The free-living amœbæ that are found in human stools may be easily differentiated from the parasitic species, during the vegetative stage of development, by the presence of a contractile vacuole in the free-living amœbæ of all species, and the structure of the nucleus with its large central karyosome. In the cystic stage the free-living species all have cysts with only one nucleus and with thicker and more irregular cyst walls. In addition, all of the free-living species may be easily cultivated upon the Musgrave-Clegg amœba agar, while none of the parasitic species can be cultivated upon this medium.

**Diagnosis of *Endamœba gingivalis*.**—This common amœba of the mouth may be easily demonstrated by making fresh or stained prepara-

#### EXPLANATION OF FIGURE 25

Protozoan cysts as they appear when stained with the iodine solution. (After Boeck. Bull. 133. Hyg. Lab. U. S. Pub. Health Service.) Explanation of figures quoted from Boeck.  $\times 1,800$ .

*Endamœba histolytica*.—Figures 12, 13, 14 (medium size race), and 15 (small size race).—Cytoplasm somewhat finely granulated, stains brown. Glycogen masses (figs. 12, 13, 15) stain dark brown; possess vague limits. Chromatoidal bodies, refractive, with rounded ends (figs. 12, 13, 14). Each nucleus represented as seen slightly out of focus, with refractive nuclear membrane, and minute central refractive karyosome.

*Endamœba coli*.—Figures 16, 17, and 18. Cytoplasm more coarsely granulated than that of *E. histolytica*. Glycogen masses with vague limits, stain dark brown, present as a rule in 1 and 2 nucleated cysts (fig. 18). Splinter-shaped chromatoidal bodies occasionally occur, but, as a rule, only in 1, 2, and 4 nucleated cysts. Inclusions are rarely present (fig. 16). Cysts contain 1, 2, 4, or 8 nuclei (sometimes 16 and 33); 8 nuclei more frequently encountered. Nuclei represented as seen slightly out of focus: each possesses a thick refractive nuclear membrane, and a larger and eccentric karyosome as a rule, compared with the minute and central karyosome of *E. histolytica*. Occasionally 2 karyosomes are present as in one of nuclei in figure 16.

*Endamœba nana*.—Figures 19 and 20. Two and four nucleated cysts. Cytoplasm stains yellow; finely granulated. Glycogen mass (fig. 19) rarely present. Several small brownish-refractive granular inclusions in cytoplasm. Nuclei, small, rather inconspicuous; possess an indistinct nuclear membrane, and a large eccentric brownish-refractive karyosome.

*Iodamœba williamsi*.—Figure 21. Cytoplasm coarsely granulated with many refractive granular inclusions. Mahogany-colored glycogen mass with sharply marked limits usually present, rarely absent. Large nucleus, with large eccentric, brownish-refractive karyosome and indistinct nuclear membrane.

*Giardia lamblia*.—Figures 22 and 23. Cytoplasm stains light brown (fig. 22) unless glycogen present (fig. 23), or in dwarf forms when it appears olive-green or bluish. Axostyles and parabasal bodies appear as refractive lines. Nuclei, 2 or 4 in number, small, usually at one end of cyst; karyosomes, central.

*Chilomastix mesnili*.—Figures 24, 25, and 26. Cytoplasm stains light brown, finely granulated, may contain a few refractive granules (figs. 25, 26). Glycogen masses staining dark brown showing vague limits, seldom encountered (fig. 24). Cytostomal fibres appear refractive (all visible in fig. 25). Nucleus round, with refractive nuclear membrane, and large eccentric karyosome (figs. 25 and 26); occasionally two chromatin masses present lying on nuclear membrane (fig. 24).

*Blastocystis hominis*.—Figures 27 and 28. Delicate vegetable organisms. The nuclei, 1 or more in number, and refractive, are located in an outer wall of cytoplasm which encloses a large vacuole, which may be empty (fig. 27) or completely or partially filled by some sort of inclusion (fig. 28).

*Phycomycete*.—Figure 29. Spores stain brown, greenish-brown, or yellow. No nuclei or other internal organs visible. The centre of the spores often stains more darkly than the outer area.



tions from scrapings from the tartar of the teeth or from pyorrhæal pus cavities, and examining such material, either unstained or stained, in the same manner as for the study of the intestinal amœbæ. The best results are obtained by wet-fixation with sublimate alcohol and staining with one of the hæmatoxylin stains, preferably the Rosenbusch stain. This amœba can be well studied in the living condition by placing a small portion of the material containing it upon a microscopic slide and covering it with a cover-glass, examining at once with a one-sixth- or one-eighth-inch dry lens.

The differential diagnosis of the parasitic amœbæ from the intestinal flagellates and vegetable cells occurring in the fæces demands a knowledge of the appearance of these various organisms and cells which can only be acquired by practice. A careful consideration of the descriptions of the morphology of the intestinal parasites and reference to the figures in the text should enable one to avoid confusing the cysts of the parasitic amœbæ with those of intestinal flagellates, or with *Blastocystis hominis* and other vegetable cells occurring in the stools, but no descriptions or illustrations can take the place of actual experience in the examination of the stools and the study of the animal and vegetable organisms which occur in this situation. No one should undertake the differential diagnosis of intestinal protozoan parasites who has not had a thorough course in the examination of the fæces of man, and who is not familiar with the common parasitic forms that may be found in the stools.

In the examination of stools for amœbæ it should be remembered that a single negative examination is of little value, and at least six or eight specimens should be examined at intervals before a negative result is accepted as final.

**Precipitin Test in Diagnosis of Infections with *Endamœba histolytica*.**—The recent work of Wagener (1924) holds out some hope for the development of a precipitin test in the diagnosis of infections with *Endamœba histolytica*. She found that uniformly positive results were obtained with an antigen prepared from scrapings of the intestinal ulcerations of cats infected with this parasite. The blood serum of cats infected for a week or longer after amœbæ appeared in the stools gave a marked reaction, while that of cats infected for a shorter time, and of normal cats, gave a negative reaction.

## CHAPTER VI

### THE PARASITIC FLAGELLATES OF MAN. THE INTESTINAL FLAGELLATES. *GIARDIA INTESTINALIS*. *TRICHOMONAS HOMINIS*. *CHILOMASTIX MESNILI*. *EMBADOMONAS INTESTINALIS*. *EMBADOMONAS SINENSIS*. *ENTEROMONAS* *HOMINIS*. OTHER INTESTINAL FLAGELLATES.

THE parasitic flagellates of man belong to the PHYLUM MASTIGOPHORA (FLAGELLATA), and include some of the most important disease-producing protozoa of man, as the trypanosomes and leishmania. Many of the lower animals also suffer from serious diseases caused by flagellates, so that these organisms are of great interest from the standpoint of both human and animal pathology. Some of the flagellates parasitic in man are apparently harmless under usual conditions, and this is true of the intestinal flagellates, which are believed by many good authorities to be harmless commensals in the human intestine.

Man is parasitized by numerous flagellates, some of which live in the intestinal canal, others in the blood, and still others in the tissues of their host. The following genera of the MASTIGOPHORA contain species that are parasitic in the body of man: *Giardia*, *Trichomonas*, *Chilomastix*, *Embadomonas*, *Enteromonas*, *Tricercomonas*, *Craigia*, *Trypanosoma*, *Schizotrypanum*, and *Leishmania*.

The biology of these parasites varies so greatly that it is considered best to consider each species separately, but for convenience of description, these parasites have been divided into two groups, i.e., the intestinal flagellates and the blood and tissue flagellates of man.

**The Intestinal Flagellates of Man.**—There are seven well-defined species of flagellates parasitic in the human intestine, some species which are regarded as doubtful, and some of coprozoic origin. The seven species which I regard as valid are the following: *Giardia intestinalis*, *Trichomonas hominis*, *Chilomastix mesnili*, *Embadomonas intestinalis*, *Enteromonas hominis*, *Tricercomonas intestinalis*, and *Craigia hominis*. It will be noted that each of these species belongs to a separate genus.

Genus I. *GIARDIA* Kunstler, 1882, Emend Alexeieff, 1914.

Synonyms: *Cercomonas*, Lambl, 1859. *Hexamita*, Davaine, 1875. *Dicercomonas*, subgen. *Dimorphus*, Grassi, 1879. *Megastoma*, Grassi, 1881.

The genus *Giardia* was established by Kunstler, in 1882, to include a flagellate of the tadpole, and there is only one species of this genus occurring as a parasite in man, *Giardia intestinalis*.

Species I. *GIARDIA INTESTINALIS* (Lambl, 1859) Alexeieff, 1914.

Synonyms: *Cercomonas intestinalis*, Lambl, 1859. *Dicercomonas muris*, Grassi, 1879. *Megastoma entericum*, Grassi, 1881. *Megastoma intestinalis* (Lambl), Blanchard, 1885. *Lambliia intestinalis* (Lambl), Blanchard, 1888. *Giardia lamblia*, Stiles, 1915. *Giardia enterica* (Grassi), Kofoid, 1920.

**History and Nomenclature.**—As indicated by the number of synonyms, the nomenclature of this flagellate is in a chaotic condition, and the exact specific name is not yet agreed upon by the majority of protozoologists. Hegner and Taliaferro (1924) accept Stiles' name, *Giardia lamblia*, but while this name is probably correct if the rules governing nomenclature be strictly applied, I believe that the name *Giardia intestinalis* is preferable as it has become fixed in the literature, and is thought by many to have as strong a position from the standpoint of nomenclatorial rules.

The genus *Giardia* was established, in 1882, by Kunstler, to include a flagellate of the intestine of the tadpole, and Alexeieff demonstrated that the species occurring in man and included in the genus *Lambliia* belonged to the same genus. The generic name, *Lambliia*, was given to this species by Blanchard, in 1888, but cannot be retained, as *Giardia*, proposed by Kunstler, in 1882, must be used according to the law of priority as applied in zoological nomenclature.

The specific name "*intestinalis*" was proposed by Lambl, in 1859. In a recent communication, Kofoid has claimed that the correct specific name should be "*enterica*," the specific name "*intestinalis*" having been previously used by Diesing, in 1850, but Dobell clearly demonstrates that the correct specific name is "*intestinalis*," and this is the name that will be used in describing this flagellate.

**Historical.**—In a very interesting contribution Dobell claims that *Giardia intestinalis* was discovered by Leeuwenhoek, the Father of Microscopy, in his own stools, and that he described the parasite in a letter to the Royal Society in 1681, but the first really clear description is that of Lambl, who named the organism *Cercomonas intestinalis*. The first accurate description was that of Grassi and Schewiakoff, which was published in 1888.

**General Morphology.**—There are two distinct stages in the life-cycle of *Giardia intestinalis*, a vegetative, motile stage, and a cystic, immotile stage, and the morphology of the organism varies greatly in the two stages. In the vegetative stage the organism is motile, pear-like in shape, and possessed of eight flagella, while in the cystic stage of development the organism is immotile, oval in shape, and flagella are not visible.

**Morphology of the Vegetative or Motile Stage.**—In the vegetative stage of development *Giardia intestinalis* is pear-shaped, the anterior end being broad and rounded, while the posterior end tapers gradually to a sharp point, known as the tail. Viewed dorsally, the organism ap-

pears arched, but when viewed laterally, a large concavity is noted at the broad, or anterior end, which is properly called the "sucking disc," roughly ovoid in shape, and which enables the parasite to attach itself to the mucous membrane of the intestine. When viewed ventrally, the "sucking disc" is seen to occupy close to three-quarters of the ventral surface at the anterior end of the organism. The younger forms are not as typically pear-shaped as the fully developed vegetative forms, being either more slender in shape or almost oval.

**Size.**—The size of the motile or vegetative phase of the organism is variously given by different authorities. The *length* of the body is given by Lambl as 18–21 $\mu$ ; Noc, as 16 $\mu$ ; Roos, as 15.5 to 17.5 $\mu$ ; Perroncito, 17 $\mu$ ; Salomon, 10 to 18 $\mu$ ; and Simon, from composite measurements based upon 342 specimens, as from 9.25 to 20.25 $\mu$ , with a mean of 13.7 $\mu$ .

The *breadth* is given by Lambl as 8.6 to 11 $\mu$ ; Noc, 10 $\mu$ ; Roos, 9 to 11 $\mu$ ; Perroncito, 10 to 12 $\mu$ ; Salomon, 7.5 to 15 $\mu$ ; and Simon, 5 to 10.25 $\mu$ , with a mean of 6.6 $\mu$ , these measurements being based upon 342 specimens.

From the above it is evident that large and small individuals of this species occur, and that the average length is about 15 microns and the breadth about 7 microns.

The great variation in the size of this species should be borne in mind in diagnosis, as otherwise the smaller individuals might be mistaken for other intestinal protozoa.

**Motility.**—The vegetative forms, when freshly passed in the fæces, are actively motile, the motion being of a jerky character, but oftentimes quite rapidly progressive. Careful focussing, using a high dry objective, will demonstrate the presence of flagella, but it is generally impossible to count them in the living specimen until motility becomes sluggish, when it is sometimes possible to detect all of these organelles. A most excellent method of studying the motility of *Giardia intestinalis* is by aid of the dark-field, and with this apparatus it is possible to study in detail the motility of each pair of flagella and the body of the organism. The dark-field shows that the body is quite rigid, but that convulsive, writhing movements occur in it while in motion, while the flagella are very flexible, with the exception of the third pair arising from the axostyles, which are not so flexible as the other flagella and wave in unison.

When the fæces are old or the organism is preparing to encyst, the active movements cease, the flagella disappear, and small pseudopodia may appear to be projected from the periphery, thus giving rise to an appearance of amoeboid motion. At this time the organism might be confused, by one unacquainted with the intestinal flagellates, with an amoebæ.

**Morphology of Stained Specimens.**—In stained preparations it is



noted that all the organelles of this parasite are paired, there being two nuclei and four pairs of flagella. The cytoplasm has a very delicate alveolar structure, and in it, at the anterior end, lie two well-defined nuclei. The nuclei lie posteriorly to the ventral depression, or "sucking disc," and directly opposite one another. They are oval in shape, and each contain a deeply staining karyosome of considerable size. The

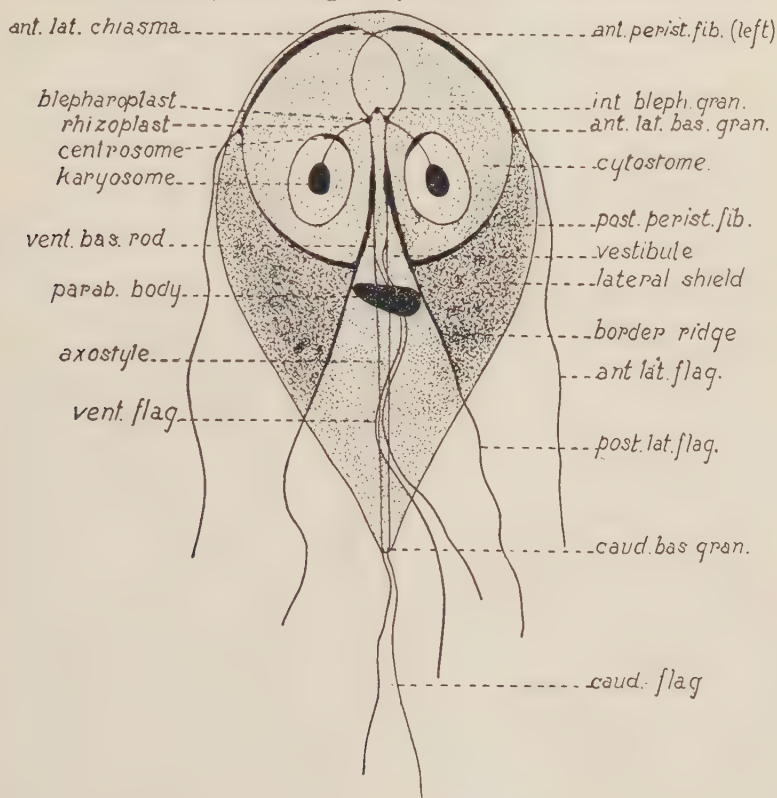


FIG. 26.—Trophozoite, or vegetative form, of *Giardia intestinalis*. (After Simon.)

chromatin in the karyosome is generally in the form of a broken, somewhat irregular mass, but in very deeply stained preparations may appear to be a solid mass. Simon (1922) states that the karyosome is connected by a very delicate linin fibril with a small dot of chromatin at the anterior pole of the nucleus, which is possibly a centrosome, but I have never been able to distinguish this appearance in the specimens that I have studied. The nucleus is contained within a well-marked, rather thin nuclear membrane, which is free from chromatin granules, and no chromatin granules can be distinguished between the membrane and karyosome.

Running the entire length of the body from a short distance back

of the anterior end, there are two rod-like, well-stained bodies, called the *axostyles*. Posteriorly these terminate in two minute granules, one for each axostyle, which are called *blepharoplasts*, while two similar granules terminate the axostyles at the anterior end. Most authorities state

that the minute granule, or centrosome, at the anterior pole of each nucleus is connected by a fine fibril with the corresponding blepharoplast at the anterior end of the axostyle, thus connecting each nucleus with the adjacent axostyle.

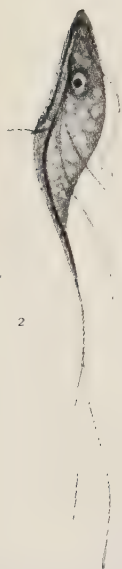


FIG. 27.—*Giardia intestinalis*. (After da Fonseca.) Iron-haematoxylin stain. 1. Ventral view of parasite. 2. Lateral view of parasite.

The *flagella* are eight in number, arranged in pairs, and are long and delicate in structure. Following Dobell and O'Connor (1921), they may be designated as the anterior, middle, ventral, and caudal pairs. The anterior

pair arise from the blepharoplast or dot at the anterior end of the axostyles, the flagella cross one another, and passing along the anterior and lateral margins of the "sucking disc," become free near the junction of the anterior and middle thirds of the body of the organism. The middle pair, arising apparently from the same origin, follow the axostyle to the posterior border of the "sucking disc" and then diverge and become free flagella near the junction of the middle and posterior thirds of the body. The ventral pair originate at the posterior edge of the "sucking disc" from the axostyles and become free near the centre of the body. This pair, as stated,

always wave in unison and are thicker and more rigid than the other flagella. The caudal pair of flagella arise from the blepharoplasts at the extreme posterior end of the axostyles, are very flexible and delicate, and become free almost at once, projecting from the tip of the posterior end or "tail" of the parasite.

It will be seen that all of the flagella arise apparently from blepharoplasts situated upon the axostyles, and that in turn the axostyles are connected with the nuclei through delicate linin fibrils. This rather complicated structure is very difficult to distinguish, even in well-stained preparations, and it is only by studying a large number of

individual organisms that the points described have been demonstrated. Still more complicated structures have been described by some authors, but it may be stated that there is no parasite in man or animals upon which the imagination of morphologists has been exercised to the extent that it has upon the structure of *Giardia intestinalis*, and much of what may be found in the descriptions of certain observers is undoubtedly imaginary, or due to artefacts.

**Morphology of the Cystic Stage.**—The cysts of *Giardia intestinalis* are very characteristic and easily recognized. They were first described by Grassi, who thought that they might be coccidia, and for a long time they were considered to be the cysts of *Trichomonas hominis*. The cysts are found in the faeces intermittently, either along with the vegetative forms or alone.

The cysts are typically oval in shape, although spherical cysts have been described by some authorities. As Dobell states, these were probably cysts viewed endwise. The cysts are colorless.

**Size.**—The cysts vary in size, and different measurements are given by different observers. Moritz and Holzl state that they measure from 10 to 13.5 $\mu$  long by 10 to 10.5 $\mu$  broad; Roos, 9.0 to 12.0 $\mu$  long by 7.5 to 10 $\mu$  broad, while Simon, as the result of the measurement of 500 cysts from five different cases, states that they are from 8 to 14 $\mu$  long by 6 to 10 $\mu$  broad, with a mean length of 10.7 $\mu$  and a breadth of 7.47 $\mu$ .

**Morphology in Detail.**—The unstained cysts of *Giardia intestinalis* are hyaline in appearance and have a well-marked refractile cystic membrane. The interior appears finely granular with minute refractile dots embedded in the cytoplasm. The cyst wall is rather thick and perfectly smooth.

The stained cysts present a very confusing structure variously described by different morphologists, and here, as in the description of the vegetative stage of development, the scientific imagination has run riot and added greatly to our uncertainty regarding what the visible structures indicate and whether some of those described were actually present or the result of faulty observation.

In stained cysts there is a definite unstained space between the cyst wall and the contents of the cysts. The majority of the cysts appear homogeneous, the cytoplasm staining faintly and appearing finely granular. In the cytoplasm may be seen the nuclei and deeply staining fibrils. If the cysts are examined shortly after encystment has occurred the flagella and axostyles may be roughly distinguished, but in the older cysts all trace of the flagella has disappeared, as well as the axostyles. The older cysts contain from two to four nuclei, the latter being frequently observed. Simon found that of 400 cysts that he studied, from

four different hosts, 96 per cent. were quadrinucleate, while binucleate cysts were rare and cysts containing more than four nuclei rarer still. Eight-nucleate cysts are sometimes observed. Multiplication must occur very promptly after encystment as binucleate cysts are so rarely observed.

The four nuclei are almost always situated at one end of the cyst, arranged in pairs, but careful focussing is necessary to demonstrate them as they do not all lie upon the same level. Cysts are rarely observed in which a pair of nuclei lie at each pole of the cyst.

The nuclei are spherical in shape, and are bounded by a well-defined

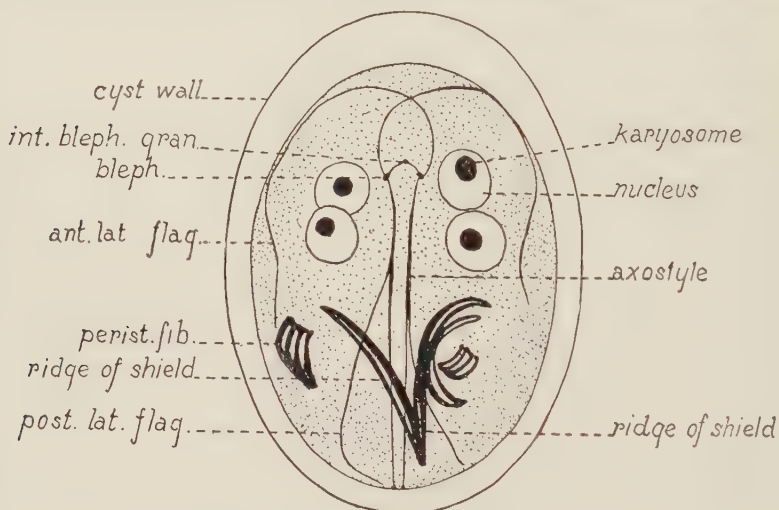


FIG. 28.—Cyst of *Giardia intestinalis*. (After Simon.)

nuclear membrane. Each nucleus contains a delicate mass of chromatin, the karyosome, which, according to Simon, may show very definite stages of division, the chromatin forming eight chromosomes arranged in an equatorial plate. I have never been able to make out these divisional phases in the karyosome of the nuclei, but have repeatedly noted that the chromatin appeared to be breaking up into delicate granules, indicating division. The nuclei are visible only in stained preparations.

Besides the nuclei, stained preparations show within the cysts deeply staining short curved fibrils apparently arranged in groups of four and two very heavily stained curved fusiform rods which form a V-shaped structure, lying near the pole of the cyst furthest from the nuclei. The nature of these structures is unknown, although some observers have not hesitated to assign to them important functions having to do with the reproduction of the parasite.

While many investigators have described other structures as visible within the cysts of this species, the descriptions are so at variance that



little reliance can be placed in them, and it may be stated that the structures that are generally visible, in well-stained specimens, are those that have been described. The size of the cysts, the presence of four nuclei at one pole, and the deeply stained fibrils and V-shaped fusiform mass, are all characteristic of the cysts of *Giardia intestinalis* and render its diagnosis comparatively easy.

With the iodine solution the cysts stain a light brown, but the nuclei do not show well in most instances.

**Habitat.**—*Giardia intestinalis* is a parasite of the small intestine of man, the active forms, or trophozoites, being most numerous in the duodenum, but occurring throughout the small intestine, down to the ileocaecal valve. The encysted forms may be found in the lower portion of the ileum, but are most numerous in the large intestine. The parasite attaches itself to the mucous membrane by means of its sucking disc, and in heavy infections considerable portions of the mucous membrane, particularly in the duodenum, may be covered with attached *Giardia*.

Under certain conditions, as in cancer or ulcer of the stomach, when the gastric secretion may be less acid than normally, *Giardia* may be found in the stomach and can be demonstrated in the vomitus or stomach contents.

The active forms, or trophozoites, are found only in the faeces when there is a fluid or semi-fluid stool, as in diarrhoeal conditions, but the cysts are found in soft or formed stools when the host is in a normal condition.

**Cultivation.**—Although efforts have been made by many investigators to cultivate *Giardia intestinalis*, the species has never been successfully cultivated.

**Species Occurring in Lower Animals.**—Species of *Giardia* are known to occur in rats, mice, guinea-pigs, rabbits, dogs, and in the tadpole. The species occurring in the mouse is *Giardia muris*, Bensen; in the meadow mouse, *Giardia microti*, Kofoed and Christiansen; in the rabbit, *Giardia duodenalis*, Davaine; in the guinea-pig, *Giardia caviae*, Hegner; in dogs, *Giardia canis*, Hegner; and in the tadpole, *Giardia agilis*, Kunstler. All of these species have been carefully studied and are believed to be distinct from the human species.

For many years it was believed that some of the species of *Giardia* occurring in the lower animals were identical with *Giardia intestinalis*, but the recent work of Bensen, Simon, and Hegner has proven that this is not true and that *Giardia intestinalis* is not a parasite of the lower animals. For long it was believed that man derived his infection from rats and mice, but this theory of infection must be abandoned in the light of the work of Simon (1922) and of Hegner (1922), who have clearly proven that the *Giardia* infesting these rodents are not identical

with *Giardia intestinalis*. The *Giardia* infesting the lower animals are all apparently harmless.

**Geographical Distribution.**—The geographical distribution of *Giardia intestinalis* is practically world-wide, although there is no doubt that infections are observed much more frequently in the tropics and subtropics, and that the infections are more severe, than in temperate regions. My own experience, covering the examination of hundreds of cases, in both temperate regions and the tropics, has convinced me that *Giardia intestinalis* is much less frequent in the northern part of the United States than in the southern part, and more frequent in the Philippine Islands than in any part of the United States. How much the frequency of occurrence of this parasite has to do with climate and how much to insanitary surroundings is a question, but I am of the opinion that the greater frequency of the parasite in man in the tropics is largely due to the poor sanitary conditions surrounding the infected. The parasite is much more frequently encountered in natives in the tropics than in the white man, and this is undoubtedly due to the greater carelessness of natives as regards sanitation.

However, it may be stated that *Giardia intestinalis* is a common parasite of man in most localities, and a survey of a large number of individuals in almost any region will result in the demonstration of instances of infection with this parasite.

**Incidence of Infection.**—For many years infections with this parasite, especially in temperate regions, were thought to be rare, but the very extensive studies of the intestinal flagellates of man made by English and American observers during the World War have demonstrated that *Giardia intestinalis* was a comparatively common parasite in soldiers of both nations. As long ago as 1915, Stiles reported the comparative frequency of *Giardia* infections in children in the United States, but his observations were not extended to adults until the examinations made during the late war.

Stiles reported the first instance of infection with *Giardia intestinalis* in the United States in 1902, and in 1915 reported the results of his examination of 1,287 school children from 6 to 17 years of age in a city of the South. Of 672 boys examined, no less than 13 per cent. were infected with this parasite, while of 615 girls, only 8 per cent. showed infection.

Kofoed (1919) and his co-workers, in their examinations of American soldiers, found that of 2,300 men serving overseas there were 131 infected with this parasite, or 5.7 per cent., while of 576 home-service men, 131 were infected, or 22.74 per cent. These men came from all parts of the United States, so that these examinations furnish a good index of the occurrence of *Giardia intestinalis* in this country.

The incidence of infection in English soldiers was higher than that of American soldiers. Mathews and Smith (1919), reporting the results of the examination of 23,024 specimens of faeces from 4,068 English soldiers convalescent from dysentery, state that *Giardia intestinalis* was found in 669 cases, or 16.4 per cent., while Jepps (1921) found the same parasite in 128 of 971 English soldiers, or 13.2 per cent. The investigations made during the World War showed that infection with this parasite was especially frequent in troops serving in Mesopotamia, Egypt, Palestine, Salonica, and the Gallipoli Peninsula.

At the Mayo Clinic, Smithies (1918) found *Giardia intestinalis* in one per cent. of 8,000 admissions, while Logan and Sandford (1916-1917) found 0.5 per cent. of infections in 1,000 admissions to the Augustana Hospital, in Chicago.

In my own experience the percentage of infections has varied greatly with locality. In the Philippine Islands the natives were found to be very commonly infected, but in the United States the percentage of infection, in my experience, has not exceeded 5 per cent. However, it is probable that in the Southern States, *Giardia intestinalis* will be found to be a very common parasite, as shown by the observations of Stiles and others.

**Life-history.**—*Giardia intestinalis* has two well-defined stages in its life-history, a motile vegetative stage and an encysted stage. The vegetative stage is spent in the small intestine of man, but when conditions become unfavorable for the existence of the vegetative stage, or when the proper developmental period is reached, the organism encysts and the cysts are voided in the faeces. In the vegetative stage of development the little animals anchor themselves to the mucous membrane of the small intestine by means of the sucking disc and absorb nourishment in this situation.

Reproduction in the active or vegetative stage is by longitudinal binary division, while a similar process of division occurs within the cysts, two individuals being thus produced. Rarely four individuals may be produced in a cyst, but this can only be considered as an atypical type of reproduction.

Infection of man occurs by swallowing food or drink containing the cysts, but the manner in which the young flagellates are liberated from the cysts and where this liberation occurs is unknown. It is believed that the covering of the cyst is dissolved by the gastric or intestinal secretions and that the young flagellates are liberated in the duodenum, as they are always most numerous in this portion of the small intestine.

**Method of Transmission.**—As already stated, the infection of man occurs through food or drink containing the cysts of *Giardia intestinalis*.

The vegetative forms are not infective so far as is known, and it is probable that they cannot withstand the acid of a normal gastric juice.

It has been believed for many years that the species of *Giardia* that are parasitic in the lower animals, especially rats and mice, were identical with the human species, and that food and drink were frequently contaminated with the cysts through rodent agency. The researches of recent careful observers negative this theory, as it has been proven that the species occurring in rodents are distinct from that of man, so that at the present time the infection of food and drink must be explained in some other way. However, it is not difficult to understand how food becomes infected where sanitary conditions are poor, and it is also possible that flies have much to do with the transmission of the infection. Stiles and Keister, in 1913, were the first to prove that the cysts of *Giardia intestinalis* could be carried by flies, and they considered that the transmission of the infection in the United States was mainly through the agency of these insects. The more recent work of Wenyon and O'Connor (1917) and of Root (1921), noted later, support the theory of insect transmission of this parasite, and undoubtedly flies have much to do with the infection of food and drink with the cysts of *Giardia*.

As the cysts of *Giardia intestinalis* are the infective agents, their resistance to external influences becomes of prime importance from a prophylactic standpoint, and some valuable data have been accumulated in this respect by students of this parasite. Wenyon and O'Connor (1917) found that the cysts of this parasite remained uninjured in the intestine of flies for as long as twenty-four hours, and that food and drink may become infected by fly droppings. Their results were confirmed by Roubaud (1918), and recently Root (1921) has proven that the cysts may remain alive in the intestine of the fly for sixteen hours, but that about half of the cysts die within eight hours. If the fly drowns in water, living cysts were found for as long as four days in the intestine, but more than half of the cysts were found dead in two days.

The presence of bacteria and the products of putrefaction in material containing the cysts causes their rapid destruction. Very interesting and important work regarding the resistance of the cysts of this species has been accomplished by Boeck (1921). He has found that the cysts will live in distilled water, at a temperature of from 12 to 22° C., for from 20 to 32 days; in distilled water in a vaseline-sealed preparation, for as long as 66 days; and that the cysts are killed only at temperatures higher than 62° C. The cysts do not withstand drying and perish quickly if exposed to direct sunlight. This fact negatives the old theory that food and drink were frequently contaminated by dust containing the cysts.

**Experimental Infection of Lower Animals.**—Success has been claimed in the infection of lower animals, especially rodents, with *Giardia intes-*



*tinalis*, and Perroncito, Bohne and v. Prowazek, Russell, Fantham and Porter, and Deschiens have all reported the successful transmission of this parasite from man to animals. Unfortunately, most of this work is of uncertain nature, owing to the fact that the experimental animals used may have suffered from infection with the species of *Giardia* common to them, and these parasites may have been confused with *Giardia intestinalis*.

Fantham and Porter (1916) were successful, they believe, in infecting seven of nine mice by feeding cysts of *Giardia intestinalis*, four of the mice dying of the infection. They state that they made repeated examination of the mice before the experiment and found them to be free from *Giardia*. Deschiens (1921) examined his experimental mice daily for two weeks before the experiment and found no *Giardia*. He then fed them material containing the cysts of *Giardia intestinalis*, and produced a severe or fatal infection in four or five animals experimented upon. Simon (1922) endeavored to infect both wild and culture rats with this parasite, but was unsuccessful in every instance, although he found that the mouse *Giardia* could be easily transmitted to the rats. As a result of these experiments and his study of the morphology of the *Giardia* of man and the mouse, he draws the following conclusion (page 424):

"Our feeding experiments thus support the conclusion we had arrived at on morphological grounds, that human giardiasis is not of rodent origin, so far as the mouse type is concerned; that the mouse type, as well as the human type, constitute separate species."

It cannot be denied that some of the experiments regarding the transmission of *Giardia intestinalis* to the lower animals are very convincing, but it is well to maintain an open mind upon this subject until more work has been accomplished, in view of the morphological studies of Bensen, Simon, and Hegner, who have apparently proven that this species does not occur in the lower animals and is distinct in morphology from any of the *Giardia* that have been found in the lower animals.

**Relation to Disease.**—Many authorities have claimed that *Giardia intestinalis* is a pathogenic parasite causing a severe and chronic type of diarrhœa in man, and some have gone so far as to describe a form of dysentery as due to this organism. A careful critical survey of the evidence offered in support of this view convinces one that much of it is untrustworthy, and Dobell and O'Connor (1921) state (page 93) that in their opinion "there is as yet no good evidence to prove that any intestinal flagellate found in man is pathogenic, but that there is very considerable evidence to show that most and probably all of them are harmless." Recently Haughwout (1918) has collected the evidence in favor of *Giardia intestinalis* being a pathogenic parasite, and, it must

be admitted, with no very favorable results so far as establishing upon scientific grounds the causative relation of this organism to any disease. Though this is so, it is true that many excellent observers have described cases in which diarrhœal symptoms were apparently due to the presence of this parasite and ceased when the parasites disappeared. Wenyon and O'Conner, during the World War, studied patients in whom attacks of severe diarrhœa occurred, accompanied by the passage of stools containing much mucus and multitudes of *Giardia intestinalis*, and in whom no other explanation of the diarrhœa was possible. They call attention to the point that it is difficult to explain the occurrence of the large amounts of mucus without admitting that it must have been produced at the localities in the intestine where the parasites were attached, and that if this is admitted, one must admit that they were the cause of the diarrhœa.

I have personally observed many instances of severe diarrhœa, especially in children, in which the stools contain mucus and even microscopic blood, and swarming with active *Giardia intestinalis*, and in which the disappearance of the organisms was followed by the cessation of the symptoms of diarrhœa. This experience has occurred too frequently, in my experience, to be merely a coincidence, and it is my belief that, under certain conditions, *Giardia intestinalis* may be of some pathogenic importance. In cases where some other cause has established an inflammatory reaction in the bowel, the irritation which must accompany the adherence of multitudes of these parasites to the mucous membrane, already inflamed, must result in an aggravation of the condition and a consequent diarrhœal condition. In other words, while I do not believe that *Giardia intestinalis*, even when present in large numbers, is the primary cause of diarrhœa, it is my belief that, given an inflamed intestine from any cause, the presence of this parasite in sufficient numbers will increase the inflammatory reaction and aggravate whatever symptoms may have been present.

Certain writers have described a form of dysentery which they considered to be caused by *Giardia intestinalis*, characterized by bloody stools and, at autopsy, by ulceration of the mucous membrane of the intestine. Such cases must have been infections with either *Endamæba histolytica* or one of the dysentery bacilli, in which this flagellate occurred as a complication, for there is no evidence that will bear careful scrutiny that *Giardia intestinalis* is capable of causing ulceration of the intestine. In my own experience, covering hundreds of infections with *Endamæba histolytica*, I have often found *Giardia intestinalis* also present, but I have never seen a case of dysentery in which this parasite occurred alone or in which a coincident infection with either *Endamæba histolytica* or dysentery bacilli could be eliminated. Whatever relation this parasite may bear

to attacks of acute or chronic diarrhoea, I am certain that it is never the cause of dysentery or of dysenteric symptoms. The frequency with which this flagellate occurs in dysentery cases is no evidence of its causal relation to the symptoms present, and a careful examination will invariably reveal that the dysentery is due to some other cause, in most instances to *Endamæba histolytica*.

**Prophylaxis.**—The prevention of infection with *Giardia intestinalis* depends upon protecting food and drink from being contaminated by the cysts of the organism. Contamination may occur either through food handlers who are “carriers” of the infection, the use of human excrement for fertilization of vegetables, the improper disposal of sewage, so that water supplies become infected, the droppings from flies that have fed upon infected fæces, and other well-known methods common where sanitation is poor or unknown. In the tropics one of the most common methods of infection is by eating salad vegetables that have been fertilized by human fæces, a common method of fertilization in many localities.

The examination of food handlers to determine whether they are infected with *Giardia intestinalis* is a valuable prophylactic measure, and no one who is so infected should be allowed to handle food or be employed in restaurants upon any duty that has to do with handling food. The protection of food and drink from flies is also a valuable measure in view of the fact, already noted, that the cysts of this parasite may remain alive in the fly’s intestine for as long as sixteen hours, and that during this time the droppings of the fly are infective. In regions where human excrement is used for fertilizing purposes all vegetables should be cooked before being eaten, and raw salads should be forbidden. In regions where sewage is properly disposed of, infections with *Giardia* are comparatively infrequent and could never reach epidemic proportions, but in localities where sewage disposal is not regulated properly this infection may assume considerable importance so far as the number of infections is concerned. Fortunately, infection is not followed by disease in most instances, so that prophylaxis is not as important or necessary as in the case of some other parasites of the intestine. However, as *Giardia intestinalis* is undoubtedly capable of aggravating any intestinal inflammatory condition that may be present or, perhaps, of rendering its host more susceptible to other and more serious bacterial and protozoal intestinal infections, prophylactic methods should be followed wherever possible.

#### Genus II. TRICHOMONAS Donne, 1837.

Synonyms: *Cercomonas*, Davaine, 1854. *Sænolophus*, Leuckart, 1863. *Monocercomonas*, Grassi, 1879. *Tetratrichomonas*, Parisi, 1910. *Pentatrichomonas*, Mesnil, 1914. *Trichomonas*, Kofoid, 1920.

The genus *Trichomonas* was established by Donne to include a flagel-

late occurring as a parasite in the human vagina, which he called *Trichomonas vaginalis*, and which also includes other species occurring in the intestine and the mouth.

### Species I. TRICHOMONAS HOMINIS, Davaine, 1860.

Synonyms: *Cercomonas hominis*, var. B, Davaine, 1860. *Cercomonas obliqua*, Moquin-Tandon, 1860. *Monocercomonas hominis*, Grassi, 1879. *Trichomonas intestinalis*, Leuckart, 1879. *Trichomonas confusa*, Stiles, 1902. *Entamæba undulans*, Castellani, 1905. *Pentatrichomonas bengalensis*, Chatterjee, 1915. *Tritrichomonas hominis*, Kofoid, 1920.

**History and Nomenclature.**—In 1854, Davaine, in examining the fæces of a patient suffering from typhoid fever, discovered *Trichomonas hominis*, but he considered it a cercomonad, and in 1860 called it *Cercomonas hominis*, variety B, to distinguish it from a similar organism which he had found in the fæces of children, and which he called *Cercomonas hominis*, variety A. Davaine gave a poor description of the flagellate, stating that it possessed only one flagellum. In 1879, Leuckart demonstrated that the organism belonged to the genus *Trichomonas*, established by Donne, in 1837. Since Leuckart's description this flagellate has been studied by numerous observers and much has been added to our knowledge of its morphology, but the large number of synonyms indicates the confusion that has existed regarding its exact name. At the present time the name *Trichomonas hominis* is quite generally accepted as the correct name of the species.

The question of the existence of more than one species of *Trichomonas* in man is still a disputed one. It will be remembered that the genus was established by Donne to include a flagellate that he found in the vagina, and some authorities still believe that this species, known as *Trichomonas vaginalis*, is identical with the species occurring in the intestine of man, as well as with those that have been found in the mouth and lungs, but careful observations have proven that these various flagellates vary in their morphology and that the species occurring in these different localities are distinct. It is believed that the evidence is now sufficient to establish at least three species of the genus *Trichomonas* as parasitic in man, i.e., *Trichomonas vaginalis*, Donne, parasitic in the vagina; *Trichomonas intestinalis*, Davaine, parasitic in the intestine; and *Trichomonas buccalis*, Goody, parasitic in the mouth. The form described as occurring in the lungs is probably identical with *Trichomonas buccalis*.

**General Morphology.**—*Trichomonas hominis* is known only in the motile vegetative stage of existence, as no one has demonstrated that this species forms cysts, as do the other intestinal flagellates. The cysts of *Giardia intestinalis*, *Chilomastix mesnili*, and other protozoa of the intestine, as well as *Blastocystis hominis*, have all, at different times been



described as the cysts of *Trichomonas hominis*, but further investigation has demonstrated their real nature, and the cystic stage of this flagellate, if such a stage occurs, is still unknown.

*Trichomonas hominis* is a small, very active flagellate possessing from three to four, and rarely five, flagella, and a very distinct undulating membrane. It is pear-shaped or roughly oval, but, owing to the plasticity of the body, the shape is constantly changing in very active specimens. When motility slows the organism becomes spherical or irregular in shape, the so-called pre-cystic forms.

**Size.**—The size is variously given by different authorities. In the living condition, Dobell and O'Connor (1921) state that the length varies from 7 to 20 microns, with a mean of 10 to 15 microns; Kofoed and Swezy (1921) give the length as from 10 to 12 microns, and the breadth as from 3 to 5 microns; while Faust (1921) states that the average length is 12 microns, and the breadth 7 microns. Most of the specimens that I have studied were from 10 to 12 microns in length and about 7 microns in breadth, but larger and smaller forms were frequently observed. In stained preparations the organism averages a little smaller, the length being about 8 to 10 microns and the breadth from 4 to 5 microns.

**Motility.**—The motility of this flagellate is very active and progressive in character and is due to the combined action of the flagella, undulating membrane, and body. In freshly passed fæces the motility is often so great that it is almost impossible to examine individual organisms, but it soon slows down and then can be studied quite easily. Apparently the organism possesses considerable penetrative power, for it can often be seen piercing its way through débris in the specimen or even through the more solid portions of the fæcal preparation. In organisms that are degenerating, or perhaps simply resting, progressive motion ceases and the border of the parasite may often be observed to be pushed out and withdrawn, although no motility results. This amœboid motion of the organism has often led to its being mistaken for a small amœba and even described as a distinct species of intestinal amœba. The organism described by Castellani as *Entamœba undulans* was undoubtedly this form of *Trichomonas hominis*.

**Detailed Morphology.**—In the living condition *Trichomonas hominis* is colorless and the cytoplasm appears finely granular. If blood is present in the fæces the cytoplasm may appear greenish in tint, due to absorbed hæmoglobin, and rarely red blood corpuscles may be present in the cytoplasm. The flagella, which arise from the anterior end, may be indistinctly seen when the organism is not very actively motile, but it is rarely that it is possible to ascertain their exact number while motility is still present. The undulating membrane is not visible in the rapidly moving organism, but when motility becomes slowed it may be distinctly

seen, appearing as a cog-wheel-like series of tiny projections at the periphery of the body, this appearance being due to the fact that one sees only the tips of the undulating waves of the membrane. The organism is roughly pear-shaped, the anterior end being rounded and rather broad, while the posterior end is pointed.

It is very difficult to obtain well-stained preparations of *Trichomonas hominis*, and it is only in such preparations that the morphological details can be distinguished. Because of this fact the descriptions of this organism are filled with inaccuracies, and some authorities have described structures that others have not been able to confirm. The best results in staining are obtained after wet-fixation with sublimate alcohol and staining with iron hæmatoxylin, but very beautiful preparations may be obtained by staining with Wright's stain, or the Giemsa stain.



FIG. 29.—*Trichomonas hominis*.  
(After da Fonseca.)  
Iron-hæmatoxylin  
stain.

In stained specimens the cytoplasm appears vacuolated and finely granular. At the anterior extremity is a small mouth, or cytostome, appearing as an unstained area near the nucleus and somewhat laterally, the undulating membrane originating upon the opposite or dorsal side of the body. A single nucleus, oval in shape, is situated at the anterior end of the body, within which is a small granular karyosome. The nucleus has a delicate nuclear membrane, and between the membrane and the karyosome minute grains of chromatin may often be observed.

At the anterior end of the body, and lying between it and the nucleus, and in very close apposition or attached to the nuclear membrane, there is a small collection of chromatin granules or masses, the blepharoplasts. In some specimens only one rather large blepharoplast is observed, while in others three or more may be present. It is from these blepharoplasts that the flagella, axostyle, and undulating membrane originate, and it is probable that there is a separate blepharoplast for each of these organelles. Faust (1921) describes the blepharoplast as (page 412) "a single spherical body, which at times is large, and composed of delicate granules, at other times drawn out into a dumb-bell shape," while Dobell (page 66) states that "at the anterior tip of the body there is a group of small blepharoplasts. It is extremely difficult to determine their precise number, but there are at least three, and probably more." I have observed the appearances noted by both authorities mentioned many times, but I believe that Dobell is correct in considering that the blepharoplasts are multiple. The single blepharoplast described by Faust is, in my opinion, the result of diffuse staining.

Arising from the blepharoplasts are from three to four flagella, which become free at once and project anteriorly. The exact number of flagella in this species is a matter of controversy, some authors claiming that only three are present, while others state that four is the normal number of flagella. Thus, Faust states that in his material only three flagella were ever observed, and that in one hundred stained specimens in which the flagella were clearly visible there was no variation from this number. Dobell states that in his experience the four-flagellate form was most frequently observed in the faeces, but that three-flagellate forms also occurred. My experience is similar to that of Dobell, as I have found that the majority of specimens of *Trichomonas hominis* have four flagella, but I have frequently seen three-flagellate forms associated with them. Forms having five flagella also occur, but are comparatively rare.

Some protozoologists consider the three-, four-, and five-flagellate forms as belonging to distinct genera. Thus Kofoed (1920) has suggested the generic name *Tritrichomonas* for the three-flagellate form, and Mesnil (1914), the name *Pentatrichomonas* for the five-flagellate form. It is very doubtful if this classification is a correct one, and at the present time it is better to consider all these forms as varieties of the same species.

The flagella are very delicate and of about the same length as the body of the parasite, and are all of nearly equal length. They originate so close together that often they appear to merge into one another and into a single blepharoplast, but in some specimens it can be seen that one or more originate from distinct granules, and it is probable that each originates from its own blepharoplast.

The undulating membrane arises from one of the blepharoplasts at the anterior end of the body and extends backward around the body in a winding manner, terminating very close to the posterior extremity of the body or the tail. The base of the undulating membrane is formed by a strong band or basal fibre, arising from the blepharoplast, while the free border of the membrane is composed of a more delicate fibril forming a flagellum. This flagellum also arises from a blepharoplast anteriorly, extends backward along the free edge of the membrane and, at the posterior end of the body, usually becomes a free flagellum of considerable length. In some instances this flagellum does not become free, but merges into the body at the posterior extremity. The undulating membrane is easily distinguished in stained specimens.

Extending from the anterior end of the body directly backward through the centre of the body is a rod-like structure, which stains hardly at all, known as the axostyle. This structure originates in a blepharoplast and extends in a straight or slightly curved direction to the posterior end of the body, where it projects, forming the sharp tail of the parasite. It is



best studied in the living specimen, where it is seen as a semirigid spine moving but slightly with the movements of the organism. Its purpose is evidently to supply support for the cytoplasm and organelles of the parasite as well as to facilitate progressive motion.

Lying in the cytoplasm are numerous food vacuoles appearing as unstained areas, varying in size. These may contain bacteria and, in rare instances, red blood corpuscles.

**Cysts.**—At this time the cystic stage of *Trichomonas hominis*, if there is one, is unknown. Even in cultures of this organism cysts have not been observed. Many observers have described bodies which they regarded as cysts of this parasite, but all have been proven to be cysts of other intestinal flagellates or amœbæ, or vegetable cells of various kinds. *Blastocystis hominis* has repeatedly been mistaken for the so-called cysts of *Trichomonas hominis* by excellent observers, as have the cysts of *Endolimax nana*. It cannot be said, however, that there is not a cystic stage of this parasite, for cysts are formed by some other species of *Trichomonas*. Dobell, in 1909, described the cysts of *Trichomonas batrachorum*, and cysts of the trichomonad of the guinea-pig, *Trichomonas caviæ*, were described by Brug, in 1917.

Degenerating forms of *Trichomonas hominis* have been mistaken for cysts, for such forms lose their flagella and undulating membrane and become oval or spherical in shape.

**Habitat.**—*Trichomonas hominis* has been found in all parts of the intestine of man, but occurs most frequently in the colon and the lower portion of the small intestine. It is apparently able to exist in any portion of the intestine, but it is more a parasite of the large than of the small intestine. As already stated, closely related species occur in the vagina and mouth.

**Life-history.**—But little is known of the life-history of this species. It is apparently very simple, reproduction occurring by longitudinal division of the parasite, which is very rarely observed. The changes which occur during division have not been worked out thoroughly, but the flagellate divides longitudinally into two parasites, the nucleus, undulating membrane, and flagella splitting as in the trypanosomes. No resting stage or resisting cysts have been demonstrated.

**Cultivation.**—In 1913, Escomel claimed to have cultivated this flagellate, but his cultures were obviously contaminated, and it is believed that the first successful cultivation of *Trichomonas hominis* was by Lynch, in 1915, who succeeded in cultivating it in beef broth. In 1918, Boyd was apparently successful in cultivating the organism in a mixture of fæces and normal salt solution. Prior to this time, in 1917, Ohira and Noguchi had successfully cultivated *Trichomonas buccalis*, the trichomonad found in the human mouth, in equal parts of ascitic fluid and Ringer's solution, while in 1920, Pringault, using the same medium, was



able to cultivate *Trichomonas hominis*. Kofoed, in 1921, obtained cultures of this flagellate in sterile cereal-lettuce infusion and in human ascitic fluid diluted with ten volumes of normal salt solution.

The best results in the cultivation of this organism appear to have been obtained by Hogue, who, in 1921, obtained pure cultures and carried them on for several weeks in a special medium composed of hens' egg and Locke's solution, and her work has been confirmed and extended by Hegner and Becker (1922), who found that *Trichomonas hominis* can be easily cultivated from an infected stool upon Hogue's medium, and that the cultures can be maintained indefinitely upon this medium.

From the observations of Hogue, and of Hegner and Becker, it is now certain that *Trichomonas hominis* has been cultivated and that the cultivation of this species of flagellate presents no great difficulties provided the proper medium is employed.

Only the motile, vegetative forms of the parasite are seen in the cultures and no cysts have ever been observed. This fact is very significant, for if cyst formation occurred in this species one would expect to observe cysts in cultures.

**Species Occurring in Lower Animals.**—The recent observations of Pringault (1920) apparently demonstrate that *Trichomonas hominis* is unable to exist in the intestine of lower animals. However, parasites belonging to the genus *Trichomonas* occur in some of the lower animals, and some of them are very like *Trichomonas hominis* in morphology. The most common species encountered in the lower animals are *T. muris*, in rats; *T. suis*, in pigs; *T. caviae*, in guinea-pigs; *T. columbarum*, in birds; *T. lacertae*, in reptiles; and *T. augusta* and *T. batrachorum*, in



FIG. 30.—Intestinal flagellates of the rat as they appear in the living condition. 1. *Giardia muris*; 2. *Hexamitus muris*; 3. *Trichomonas muris*.  $\times 2,500$ . (After Hegner.) Note. This illustration is included in order to show the appearance of the intestinal flagellates in the unstained, living condition.

amphibians. None of these flagellates are pathogenic to their hosts, so far as has been ascertained.

**Geographical Distribution.**—*Trichomonas hominis* has a world-wide distribution, but is more frequently observed in the subtropics and tropics than in temperate regions. It occurs as a comparatively common parasite in the southern part of the United States, and during the World War was found not infrequently in soldiers returning from France. In the Philippine Islands I found a large proportion of native children harboring this parasite without apparent injurious effect, and it was also a common parasite of adults.

**Incidence of Infection.**—This species, like other species of intestinal flagellates, is most common where sanitary conditions are poor, and it therefore follows that the highest percentages of infection will be found in native races in tropical regions, while in the white man, in our modern cities, the incidence of infection will be low. However, even in natives, it is not so common a parasite as *Giardia intestinalis* or *Chilomastix mesnili*, and the observations of many competent investigators have demonstrated that *Trichomonas hominis* is a comparatively rare species of intestinal flagellate in most localities.

During the World War the incidence of infection with this flagellate was carefully studied by several observers. Kofoed, Kornhauser, and Plate, in 1919, examined 2,400 American soldiers who had been invalided from France and found only three infected with this parasite, or 0.1 of 1 per cent., while of 576 home-service men, the same number was found infected, or 0.5 of 1 per cent. In a later series of observations, made at the University of California, in 1920, Kofoed examined 91 students who had served overseas and found one infected, or 1.1 per cent., while 34 students who had served only at home showed two infections, or 5.8 per cent. Mathews and Smith, in 1919, made 23,924 examinations of fæces from 4,068 convalescent English soldiers, most of whom had suffered from dysentery, and found 29 infections with *Trichomonas hominis*, or 0.7 per cent., while Jepps, in 1921, examined 971 English soldiers at the Southampton Hospital and found only 12 infections, or 1.2 per cent. In China, Faust examined 359 patients in hospital at Wuchang and found this parasite in 0.6 of 1 per cent.

From these observations it is evident that in temperate regions *Trichomonas hominis* occurs in from one-half to 1 per cent. of individuals examined, but in the tropics the percentage is much higher, especially among the natives where sanitation is poor. In such regions, in my experience, as high as 10 per cent. of the children may be infected in certain localities. Fletcher and Jepps (1924) examined 1,034 Asiatics in the Federated Malay States and found 119 infected with this parasite, or 11.9 per cent.

**Method of Transmission.**—So far as we know *Trichomonas hominis*

is transmitted to man through the agency of food and drink contaminated by the vegetative form or trophozoite. In the absence of definite knowledge as to the existence of a cystic stage in the life-history of this parasite we must believe that the motile, vegetative form is infective, although this is not in accordance with what is known of the vegetative forms of other intestinal flagellates, which are unable to withstand the action of the normal gastric secretion, and, therefore, are not infective.

The occurrence of cysts in the trichomonads of some of the lower animals, as the guinea-pig, suggests that there may also be a cystic stage in the development of *Trichomonas hominis* which has escaped observation. Such a stage in development may occur outside of the human body, and this may explain why cysts have not been observed, as they may not be formed in the faeces for some time after they are voided or under unknown and peculiar conditions.<sup>1</sup>

Little work has been done upon the resistance of *Trichomonas hominis* to external conditions. In stools kept at a temperature of 37° C. and diluted with distilled water the organism may remain alive for as long as three weeks, in my experience, and Dobell states that in some liquid stools it will remain alive for as long as one month. The fact that cysts have never been observed even when the stools containing the parasite are a month old and the parasite is still alive, is, in my opinion, almost positive proof that there is not a cystic stage of development.

Woodcock (1917) has shown that *Trichomonas hominis* will remain alive for five and a half hours in 0.066 hydrochloric acid solution, and Wenyon (1915) claimed that the round, immotile forms can resist the action of the normal gastric secretion for a considerable time. These observations demonstrate that the organism can, in all probability, pass through the stomach without harm, and that infection can occur without the necessity of a cystic stage of development.

Some authorities have claimed that transmission of this parasite may occur through the air, but this is most improbable, nor do flies act as transmitters so far as is known, although such a method of transmission might occur by food becoming contaminated by material carried on the feet or body of these insects. However, the fly is certainly not so often a transmitter of this flagellate as of those that form cysts which pass unharmed through the insect's intestines and reach food or drink in the droppings. There is no evidence showing that *Trichomonas hominis* may pass unharmed through the intestine of the fly.

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<sup>1</sup> Hegner (*Am. Journ. Hyg.*, 1924, iv, 143) has shown, by experiments on rats, that *Trichomonas muris*, the parasitic trichomonad of this animal, when fed to rats in food, passes unharmed through the intestinal tract in the flagellate stage of development. He found that infection of rats followed the ingestion of the motile flagellates, which are unharmed by the digestive secretions of the stomach or intestines. These experiments indicate that the same may be true of *Trichomonas hominis*, and that a cystic stage of development is not essential to the transmission of this parasite.



**Experimental Infection of Lower Animals.**—The experimental infection of some of the lower animals with *Trichomonas hominis* has been claimed by several observers. Escomel, in 1913, claimed to have infected the dog, cat, rabbit, and guinea-pig with this parasite, but his observations have not been confirmed and are open to serious doubt. In 1915, Lynch infected a rabbit by rectal injection of material containing the parasite, and in 1919, Boyd infected a rat *per os*. On the other hand, the experiments of Pringault, in 1920, who endeavored to infect the cat, rabbit, guinea-pig, and white rat *per rectum*, were unsuccessful. It is evident that infection with *Trichomonas hominis* is not easily transmitted to the lower animals, and it cannot be said that the proof of such transmission is of such a nature as to preclude some doubt as to its reliability.

**Relation to Disease.**—Whether or no *Trichomonas hominis* is a pathogenic parasite is still in dispute, some authorities claiming that it produces diarrhoeal conditions, and even dysentery, while others regard it as a harmless commensal.

Brumpt, in 1912, described a case of colitis which he believed to be due to this parasite, and in 1913, Escomel described an epidemic of dysentery, numbering 152 cases, which he studied at Arequipa, Peru, in 1898, and which he believed to be due to *Trichomonas hominis* obtained from an infected water supply. His observations cannot be accepted as conclusive as regards the relation of this parasite to the epidemic, as the possible presence of dysentery bacilli or *Endamæba histolytica* was not eliminated, and it is more than probable that the epidemic was one of bacillary dysentery, in which the presence of *Trichomonas hominis* in the stools was purely a coincidence. Rhamy and Metts, in 1916, studied an epidemic of dysentery in which there were 78 cases with 17 deaths, and which they regarded as being caused by this organism. The description of the clinical symptoms suggests bacillary dysentery, and neither this condition nor amœbic infection was sufficiently ruled out. The fact that treatment with ipecac was followed by recovery in some of the cases indicates that they were due to amœbic infection, as ipecac is of no service in the treatment of infections with *Trichomonas hominis*, while the fatality of the epidemic certainly speaks against its being caused by this flagellate.

Instances of infection with this parasite, in which the symptoms present were thought to be due to it, have been reported by other observers, notably Chatterjee, in 1917, who considers it a cause of diarrhoea and dysentery. Wenyon, in 1920, reported finding *Trichomonas hominis* in the wall of the small intestine, but there were no ulcerations due to its presence nor evidences of inflammatory reaction, which suggests that the parasites may have reached this locality post-mortem.

The observation that *Trichomonas hominis* may sometimes ingest red blood corpuscles is not an evidence of its pathogenic nature, as urged



by some observers, for this parasite ingests other materials as food, and, if blood happens to be present in the stools, the fact that it is capable of ingesting the corpuscles is no proof that it is capable of burrowing into the intestine in search of blood. I have repeatedly observed trichomonads with ingested red blood corpuscles within them in cases of dysentery, but in every such instance either *Endamæba histolytica* has been present or some variety of the dysentery bacillus has been isolated.

A careful consideration of the claims made by those who regard *Trichomonas hominis* as a pathogenic parasite convinces one that they are not based upon definite scientific evidence, and while we cannot deny that this parasite may, when present in large numbers, aggravate an already existing lesion in the intestine, it is not, I believe, capable of exciting an attack of diarrhœa or dysentery except, perhaps, in very young children, and even then it is doubtful if this parasite really initiates the condition. These flagellates are undoubtedly more numerous in diarrhœal stools than in normal stools, but this is no proof of their etiological relation to the condition present, for it is well known that in healthy individuals the administration of a cathartic is often followed by the appearance of *Trichomonas hominis* in the stools when previous examinations of the fæces had been negative for this parasite.

At the present time it must be admitted that the evidence available is not sufficient to prove that *Trichomonas hominis* is a pathogenic parasite.

### Genus III. CHILOMASTIX Alexeieff, 1910

Synonyms: *Cercomonas*, Davaine, 1854. *Trichomonas*, Leuckart, 1870. *Monocercomonas*, Epstein, 1893. *Macrostoma*, Alexeieff, 1909. *Tetramitus*, Alexeieff, 1910. *Fanapepea*, Prowazek, 1911. *Cyathomastix*, Prowazek and Werner, 1914.

#### Species I. CHILOMASTIX MESNILI (Wenyon, 1910) Alexeieff, 1912

This flagellate has been frequently rediscovered and renamed, so that the synonyms are very numerous. The most important are the following:

Synonyms: *Cercomonas*, var. A, Davaine, 1854. *Cercomonas davaini*, Moquin-Tandon, 1860. *Cercomonas intestinalis*, Marchand, 1875. *Trichomonas intestinalis*, Leuckart, 1879. *Monocercomonas hominis*, Grassi, 1881. *Macrostoma mesnili*, Wenyon, 1910. *Tetramitus mesnili*, Alexeieff, 1910. *Fanapepea intestinalis*, Prowazek, 1911. *Cyathomastix hominis*, Prowazek and Werner, 1914. *Chilomastix davaini* (Moquin-Tandon), Kofoid, 1920.

**History and Nomenclature.**—The number of synonyms of this flagellate is sufficient evidence of the confusion which has existed regarding its exact generic and specific position. It was probably first seen by Davaine, in 1854, who regarded it as a cercomonad and later named it *Cercomonas hominis*, var. A. In 1871, Cunningham described it and called it *Cercomonad B*, while in 1875, Marchand called it *Cercomonas intestinalis*. In 1910, Wenyon described the organism more fully, recognized that it was not a cercomonad, and called it *Macrostoma mesnili*, but the name

*Macrostoma* had already been used by Alexeieff and was no longer available. In 1912, Alexeieff proposed the generic name *Chilomastix* for the organism and this is now accepted as the correct generic name.

The specific name *mesnili* was given the flagellate by Wenyon, in 1910, but Kofoid, in 1920, called attention to the fact that Moquin-Tandon, in 1860, had renamed Davaine's *Cercomonas hominis*, var. A, *Cercomonas davainei*, thus making the specific name "*davainei*" the proper specific name for *Chilomastix mesnili*, if the law of priority is strictly followed. However, the name *Chilomastix mesnili* has become so firmly fixed in the literature that it should be retained, as this is an instance in which general usage is of more weight than a hide-bound adherence to the rules of nomenclature.

**General Morphology.**—*Chilomastix mesnili* has a vegetative and cystic stage in its life-cycle. The vegetative form, or trophozoite, is an asymmetrical, pear-shaped organism having a rounded anterior end and a sharp-pointed posterior extremity, or tail. There are four flagella, three directed anteriorly and one, situated within the mouth, directed posteriorly. The body is deeply grooved in a spiral direction and is characterized by a very large mouth, or cytostome, situated near the anterior end. The cysts of this species are characteristic, being oval in shape with a small, blunt projection at the anterior end, giving them a lemon-like appearance.

**Morphology of the Vegetative Form or Trophozoite.**—In the living condition the vegetative or motile form of *Chilomastix mesnili* is very apt to be mistaken for *Trichomonas hominis*, and some observers have mistaken it for *Giardia intestinalis*, but attention to the morphology of the parasite should obviate such errors.

**Size.**—The size of the parasite is variously given by different authorities, but individual organisms vary much in size in the same specimen. According to Kofoid and Swezy (1921), the living trophozoites are from 13 to 24 microns in length with an average length of 19.6 microns. Dobell (1921) gives the length as between 6 and 20 microns, but usually between 10 and 15 microns, while Boeck (1921) states that in the material that he examined the organisms varied between 3 to 19 microns in length and 2 to 9 microns in width, but that most of the forms measured from 8 to 14 microns in length.

Stained specimens are smaller, those usually observed measuring 7 to 12 microns in length.

**Motility.**—This flagellate is actively motile in freshly passed fæces, the general character of the motility being progressive. In old specimens of fæces the motility is generally very sluggish, at which time it is possible to demonstrate that long after the free anterior flagella have ceased motion the flagellum situated within the mouth is still actively moving, its undulations resembling those of an undulating membrane,

and some observers regard this flagellum as forming the upper margin of such a membrane. The organism moves in a rather jerky manner either forward or in a spiral direction and apparently by means of the three anterior flagella, the flagellum within the mouth serving only as a means of directing food particles into the mouth.

**Body and Contents.**—In the living condition the body is colorless,

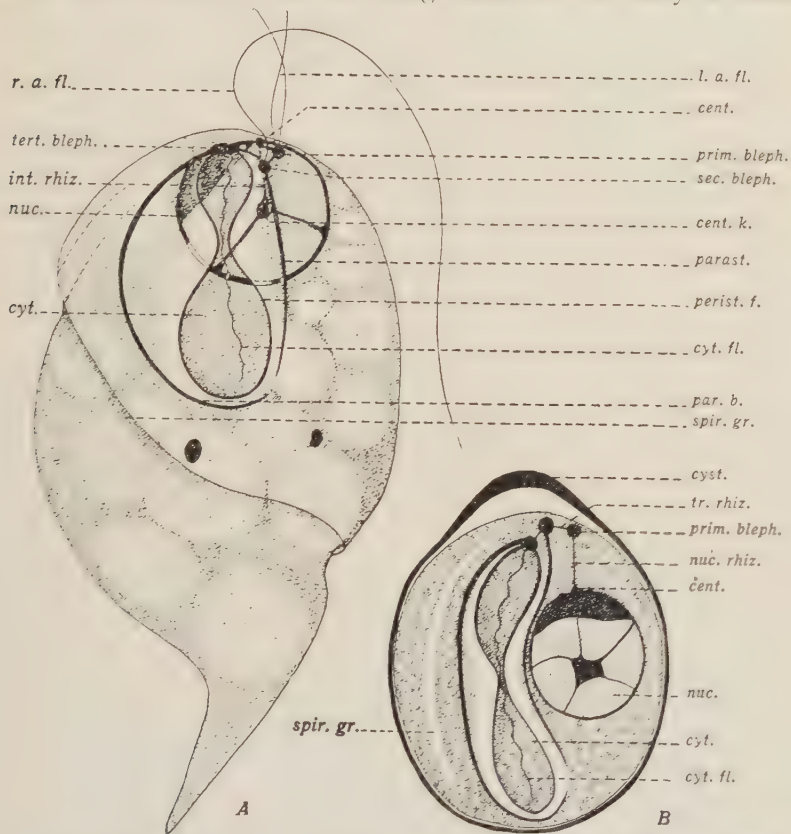


FIG. 31.—Trophozoite and cyst of *Chilomastix mesnili*.  $\times 6,370$ . (After Kofoid and Swezy.)  
A. Normal *C. mesnili* viewed from the ventral or oral side and showing all the structures of the body.  
B. Cyst of *C. mesnili* viewed from the ventral or oral side.

or of a faintly greenish tint, and the cytoplasm appears finely granular and contains numerous hyaline spherical areas, the food vacuoles. The body in some of the organisms appears to be twisted upon itself, but it is doubtful if this is a natural condition. The flagella and cytostome are distinguishable with some difficulty, but for the study of these structures and the finer details of the morphology of the parasite stained preparations are necessary. Wet-fixation and staining with iron hæmatoxylin gives the best results.

In stained preparations the body is roughly pear-shaped, the anterior

end being broad and rounded, while the posterior is attenuated to a sharp-pointed tail which may be prolonged into a flagellum-like thread. The body tapers quite rapidly to the pointed tail, but not equally upon each side, so that the body, as a whole, is asymmetrical. The *cytoplasm* appears dimly stained and finely granular, and contains numerous food vacuoles.

The most conspicuous object within the body of *Chilomastix mesnili* is the large *cytostome*, or mouth, which begins at the anterior end upon the ventral aspect of the organism and extends backward, in a spiral direction, for nearly one-half the length of the body. It appears as a cleft in the cytoplasm and has two well-marked lips, the right lip being larger and more easily observed than the left. Lying within the mouth is a short flagellum which, according to Kofoed and Swezy (1920) and Boeck (1921), forms the upper margin of an undulating membrane, but which Dobell (1921) believes to be a free flagellum. My own observations are in agreement, as a whole, with those of Dobell, but I have seen organisms in which the flagellum moved in such a way as to make the resemblance to an undulating membrane very striking. The flagellum within the mouth is called the buccal flagellum, and in life is in constant undulatory motion.

The body is marked by a *spiral groove* running in a diagonal direction entirely around the organism, from the dorsal side of the anterior extremity to the ventral side posteriorly. This groove is well marked in most individuals, but may be poorly defined, or even absent, in some. Kofoed and Swezy (1920) regard it as a contractile area of the body which varies in depth at different times.

Lying in the cytoplasm near the cytostome and at the anterior end of the body is a spherical or oval *nucleus*, having a well-stained nuclear membrane. There is a minute karyosome situated eccentrically, and minute granules of chromatin may be observed between the nuclear membrane and the karyosome. Traces of a linin net-work may be seen within the nucleus and, in some instances, the karyosome may be double or consist of a mass of loosely arranged granules. The contents of the nucleus are so minute that it is often impossible to make out the exact arrangement, and it is only in very well stained specimens that the structures mentioned can be distinguished.

At the anterior pole of the nucleus and, apparently, in contact with the nuclear membrane, there lie a group of minute, deeply stained granules or *blepharoplasts*. It is very difficult to distinguish the number of these, but Dobell (1921) states that there are six arranged in a circle, while Kofoed and Swezy (1920) give the number as three. The latter authors describe a very tiny centrosome at the anterior pole of the nucleus, but Dobell denies that a centrosome can be recognized, and it



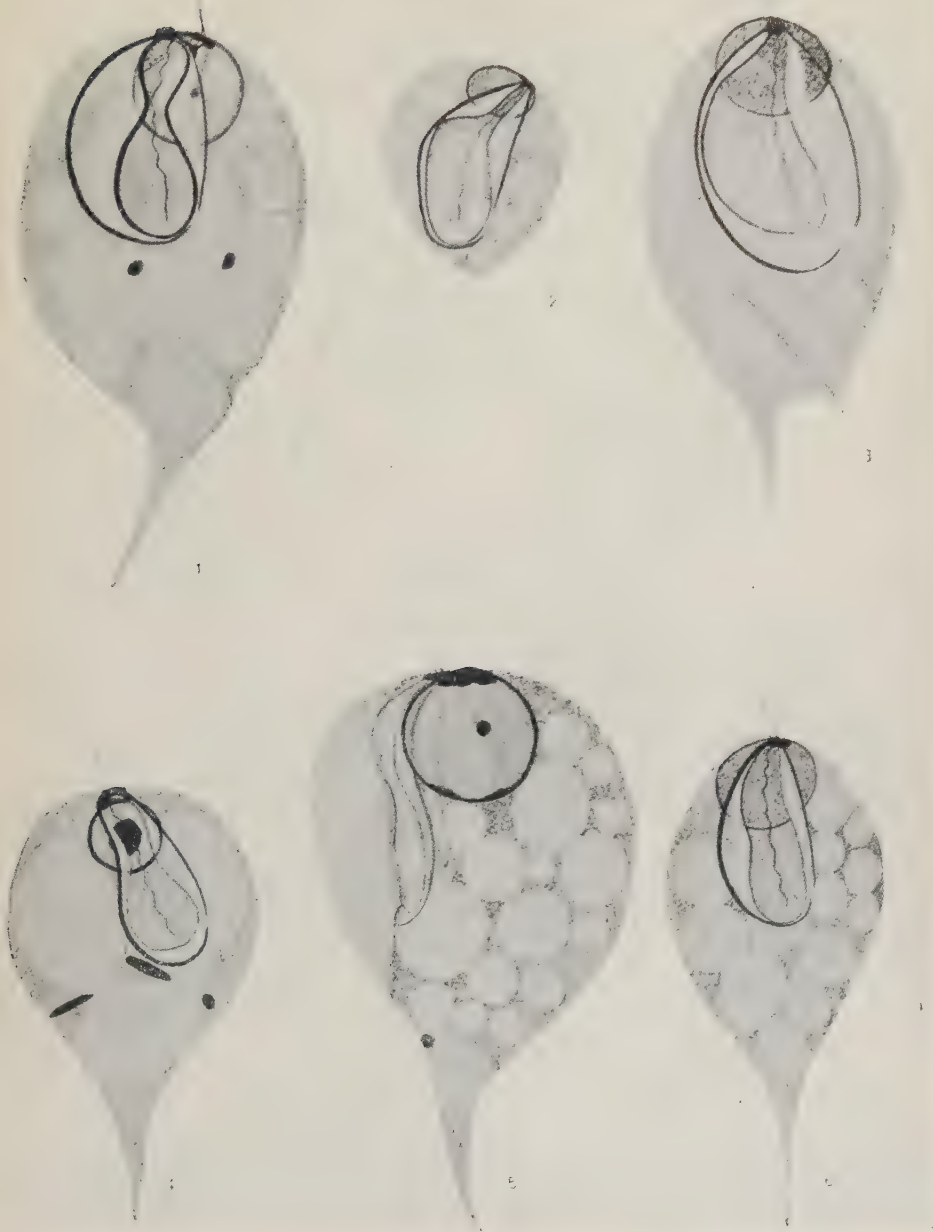


FIG. 32.—*Chilomastix mesnili*.  $\times 4,800$ . (After Kofoed and Swezy.) Iron-haematoxylin stain. 1 to 6. Various forms of the trophozoite of *Chilomastix mesnili*. In 1 the spiral groove, the cytostome with its peristomal fibre, cytostomal flagellum, parabasal and parastyle, the nucleus, and the three blepharoplasts and centrosome are all shown.

is difficult to understand how it could be differentiated from the mass of small granules or blepharoplasts, as it is only in very finely stained preparations that even these can be distinguished.

Arising from the group of blepharoplasts there are three *flagella* which become free at once and are directed anteriorly, and, in addition, according to Dobell, a fourth flagellum which is shorter and directed backward within the mouth or cytostome, and two fibrils, one supporting the right lip of the buccal cavity and another supporting the left lip of this cavity. The three anterior flagella are of equal length, while the posterior flagellum is much shorter and more delicate.

The undulating membrane described by Chalmers and Pekkola (1918), Kofoid and Swezy (1929), and Boeck (1921) answers in its morphology to the flagellum within the cytosome or mouth of the parasite, and careful examination of many specimens has convinced me that it is this structure that has been interpreted by these authors as an undulating membrane.

**The Cysts.**—The cysts of *Chilomastix mesnili* are very characteristic lemon-shaped bodies having a short, blunt projection at the anterior or smaller end. Spherical, ovoid, or asymmetrically shaped cysts also occur, but the vast majority of them are typically lemon-shaped.

**Size.**—The size of the cysts varies considerably. Dobell gives the average measurements as 7.6 to 8.5 microns in length; Kofoid and Swezy state that the length varies from 6.5 to 9 microns, with an average of 7 to 7.5 microns, and the breadth from 5.8 to 7.5 microns, with an average of 6.5 microns; Boeck gives the length of the cysts as from 7.6 to 9 microns, and the breadth as from 4.5 to 6 microns. Larger cysts may occur, but are very rare.

**Morphology of Unstained Cysts.** In unstained specimens the cysts are colorless, the cyst wall being transparent and thickened at the smaller, or anterior end, where the projection, already noted, occurs. The contents of the cyst appear finely granular and bright, refractive granules are often noted within the cysts. With the iodine solution the contents usually take a diffuse yellowish stain, but some of the cysts may show a deep-brown mass within them, the glycogen vacuole, but this is an unusual appearance, the cyst, if it contains glycogen, staining a uniform brownish color with the iodine solution.

**Morphology of the Stained Cysts.** In stained specimens the cytoplasm appears finely granular and a distinct *nucleus* is present, of large size and lying at the anterior or small end of the cyst, or near the middle in the older cysts. The chromatin of the nucleus is usually condensed in a large mass at some portion of the periphery of the nucleus upon the nuclear membrane or it may be present as a large central karyosome.

The nuclear membrane is well defined and may or may not be covered internally with a row of chromatin granules.

Besides the nucleus the cysts of this species contain a peculiar structure, the remains of the buccal cavity or *cytostome*. The fibrils supporting the lips of the cytostome, already mentioned, are still preserved in the

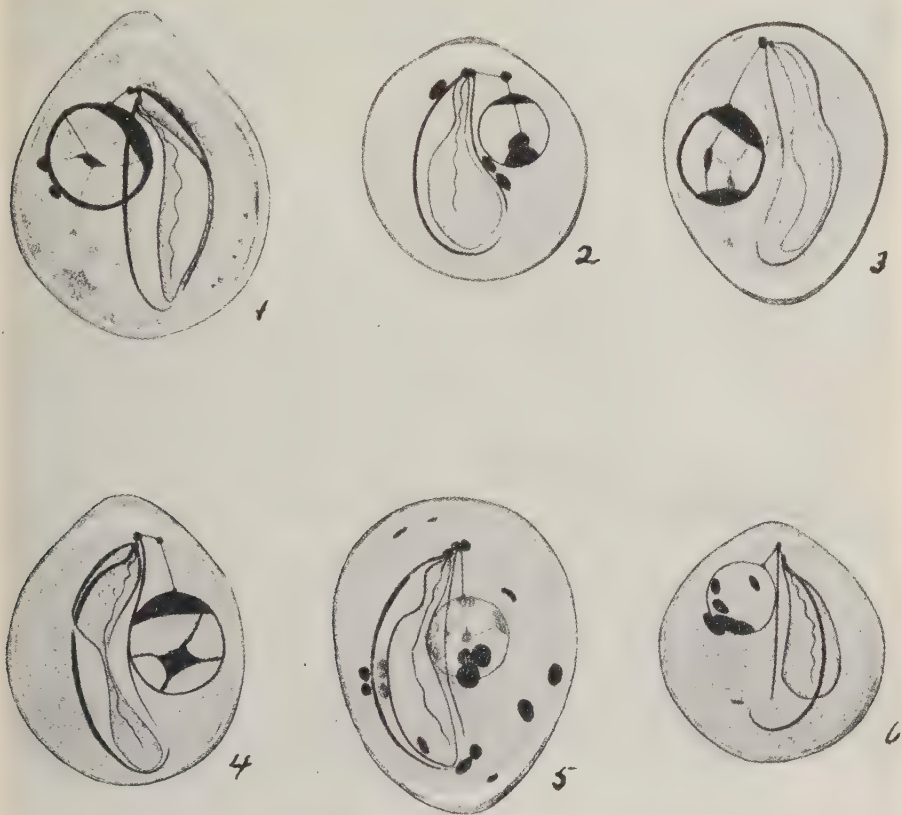


FIG. 33.—*Chilomastix mesnili*.  $\times 4,800$ . (After Kofoid and Swezy.) Iron-haematoxylin stain. 1 to 6. Various forms of the cysts of *Chilomastix mesnili*. 1. Typical cyst with wall at anterior end thickened. 2. Spheroidal cyst with large chromidial bodies. 3. Typical cyst. 4. Cyst containing many chromidial bodies. 5. Organism inverted within the cyst. 6. Subspheroidal cyst.

cyst, and the one supporting the right lip extends backward to nearly the posterior end of the cyst in a loop-like manner, and joining the fibril supporting the left lip. Within the area outlined by these fibrils may be seen the posterior flagellum as in the vegetative form or trophozoite.

The blepharoplasts from which the three anterior flagella arise, as well as those from which the buccal fibrils and posterior flagellum originate, may be seen within the cysts appearing as deeply stained dots or

granules lying free in the cytoplasm and detached from the nuclear membrane, but in rather close association with the nucleus.

Kofoid and Swezy (1920) describe a division of the parasite within the cyst into two organisms, but I have never seen a cyst of *Chilomastix mesnili* which contained more than one nucleus. In regard to division within the cyst, Dobell (1921) (page 78) says: "Although we have kept cysts for several weeks, until they finally degenerated and died, and, although we have examined thousands upon thousands of cysts in human



FIG. 34.—Camera lucida drawings of dividing forms of *Chilomastix mesnili* in cysts and of dividing nuclei of *C. mesnili*. (After Hegner.)  $\times 3,500$ . 1 to 3. Cysts, each containing two nuclei, several blepharoplast-like bodies, and a number of fibrils. 4 to 9. Nuclei in various stages of mitosis.

fæces, we have never yet seen a single cyst containing more than one nucleus. If nuclear division does occur within the cyst it must be extremely rare."

The recent investigations of Hegner (1923) prove beyond doubt that nuclear division does occur in the cysts of *Chilomastix mesnili*, although it has not been observed by Dobell and myself. Binucleate cysts were observed in some of his preparations, and his figures show conclusively that nuclear division in the cysts occurs by mitosis. Hegner does not state just how often he has observed cysts containing two nuclei, but does state that in some of his preparations they were numerous. The results of Hegner show how easily erroneous conclusions may be drawn from the experience of single observers and that, in the study of the Protozoa, one cannot be too dogmatic regarding the work of others when judged by personal experience.

The cysts of *Chilomastix mesnili* were first fully described by Wenyon and O'Connor (1917), and Dobell and Jepps (1917).

**Habitat.**—This flagellate is a parasite of the intestine of man, but



there is still considerable uncertainty as to whether it inhabits the large or small intestine, or both. Dobell and O'Conner (1921) claim that it lives in the large intestine, but they do not deny that it may also occur in the small, while Boeck (1921) states that it is a parasite of the small intestine. Hegner and Taliaferro (1924) believe that it lives principally in the large intestine, but the appearance of the cysts only in formed stools, and of the vegetative forms only in liquid stools after a cathartic, or in diarrhoeal conditions, is suggestive of its being located mostly in the small intestine. That the vegetative forms do occur in the large intestine at times, at any rate, has been demonstrated by Wenyon, who found them in the tissues of this portion of the intestine. It is probable that *Chilomastix mesnili* is able to live in any part of the intestine of man, but that it is preferably a parasite of the large intestine.

**Species Occurring in Lower Animals.**—Species of *Chilomastix* have been described as occurring in the goat, rabbit, guinea-pig, rat, and fowls, and those that have been well studied, as *Chilomastix intestinalis*, of the guinea-pig, and *Chilomastix caulleri*, of the tadpole, have been found to be distinct from *Chilomastix mesnili*, which, so far as is known, does not occur in any of the lower animals.

Other species of *Chilomastix* occurring in lower animals are *C. cuniculi*, of the rabbit; *C. capræ*, of the goat; *C. bittencourti*, of the rat; and *C. motellæ*, of fish. These species apparently differ considerably in morphology from *Chilomastix mesnili*, and are undoubtedly distinct from the latter species.

**Cultivation.**—The first successful attempt to cultivate this flagellate was that of Boeck (1921), who reported that he had been able to cultivate it in a mixture of human blood-serum and Locke's solution. In tubes containing this mixture it was possible to keep the organisms alive for from 2 to 10 days, the average life of a culture being from 6 to 8 days. During this time the flagellates multiplied, reproduction occurring by longitudinal fission generally, although multiple division also occurred, four flagellates being produced. Multiplication continued for as long as four days and then the number of flagellates in the cultures gradually decreased. Cysts were not observed in the cultures. Boeck maintained the cultures for a period of six months.

The culture results of Boeck with this species were confirmed by Hegner and Becker, in 1922. They found that *Chilomastix mesnili* grew well in a medium composed of a mixture of white of egg and 0.7 per cent. saline solution, and that it could be obtained in cultures from the fæces when the latter were negative microscopically, and they recommend this culture method as being superior to microscopic examination in diagnosis.

In the hands of an expert such a method of diagnosis would doubtless

be very efficient, but it is not believed that in ordinary hands it can replace a careful microscopic examination of the fæces.

**Life-history.**—*Chilomastix mesnili* has two stages in its life-cycle, a vegetative, motile stage, and an encysted stage. During the vegetative stage it takes nourishment through the mouth, or cytostome, the food apparently consisting largely of bacteria, which, after passing through the mouth, are digested in the food vacuoles with which the cytoplasm is often filled. Reproduction occurs by longitudinal, binary division, but dividing forms are rarely observed in the stools. In the cystic stage of development reproduction does not occur, according to the vast majority of observers. The cyst is evidently a resistant form of the flagellate which transmits the infection from host to host.

Nothing is known as to the length of life of either the vegetative or cystic form under natural conditions.

**Geographical Distribution.**—*Chilomastix mesnili* has a world-wide distribution and has been reported from many different countries. It is more frequently encountered in the tropics and subtropics than in temperate regions, but it is not a tropical species, being widely distributed throughout Europe and the United States.

**Incidence of Infection.**—Important additions to our knowledge of the incidence of infection with this flagellate were made during the World War and we know that, next to *Giardia intestinalis*, it is the most common flagellate occurring in the intestine of man. Kofoed and Swezy (1921) report that of 2,300 American soldiers who served overseas, 97, or 4.2 per cent., were infected with this organism, while of 576 home-service men, 20, or 3.5 per cent. showed infection. In an examination of students at the University of California, in 1920, the same investigators found that of 534 individuals, 28 harbored this flagellate, or 5.3 per cent. Mathews and Smith, examining 4,068 English soldiers, found 148 infections with *Chilomastix mesnili*, or 3.6 per cent. Their studies demonstrated that the percentage of infection varied greatly in different classes of patients. Thus, dysenteric patients showed from 12 to 15 per cent. of infections, amœbic patients showing 14.2 per cent.; insane patients showed 23.2 per cent. of infections; while in 548 infirm children only 1.8 per cent. were infected, and in 450 routine surgical patients in civil hospitals the rate of infection with this flagellate was less than 2 per cent.

In my own experience the incidence of infection with this parasite has not exceeded three to four per cent. except in patients suffering from diarrhœal conditions. I have noted that this flagellate is very frequently associated with *Endamœba histolytica*, and when it is present, a prolonged search for the latter parasite is often rewarded by success, even though symptoms of dysentery may not be present.

**Method of Transmission.**—Man becomes infected with *Chilomastix mesnili* through food or drink contaminated with the cysts of the flagellate. Wherever sanitary conditions are poor, and the disposal of sewage is faulty, food may become easily contaminated. This may occur directly through infected food handlers, or indirectly through the use of human excrement for fertilizing garden vegetables. The comparatively large number of infections among the insane is evidence of the facility with which it is transmitted from person to person where personal hygiene is neglected.

The transmission of this flagellate by means of flies, either through contamination of food or drink by cysts which have adhered to the body of the fly, or through fly droppings, is probably a common method of infection. Root, in 1921, demonstrated that both motile and cystic forms of this flagellate could pass through the intestine of the common house-fly and the ordinary blow-fly unharmed. He found that the motile forms, within the intestine of the fly, died within an hour, and that encystment did not occur, but that they could be found in a motile condition in the fly's faeces as early as seven minutes after feeding on a stool containing them.

Root found that the cysts were much more resistant, remaining alive in the fly's intestine for as long as 80 hours, although about half were found dead within 36 hours, and that they remained alive longer in the intestine of the fly than the cysts of any other species of protozoan parasite of the human intestine.

The resistance of the cysts of *Chilomastix mesnili* to certain external agencies has been investigated by Boeck (1921). When placed in distilled water and kept at a temperature between 12 and 22° C., the cysts remained alive for 187 days, and in wet preparations in distilled water, sealed with vaseline, and kept at the same temperature, they remained alive for 232 days, a period far in excess of the survival of the cysts of other protozoa occurring in the intestine of man.

Boeck (1921) determined, in another series of experiments, that the thermal death point of the cysts of this flagellate is 72° C., and that at temperatures between 50° and 60° C., the cysts were much more resistant than those of *Endamæba histolytica*, *Endamæba coli*, or *Giardia intestinalis*.

The cysts are killed quickly by exposure to direct sunlight, and by drying, and they can remain alive in the faeces only as long as the latter are in a moist condition. Therefore, the transmission of this organism by dust is impossible and the air does not convey the cysts to food or drink.

**Experimental Infection of Lower Animals.**—There is no well-authenticated instance of the experimental transmission of *Chilomastix mesnili*

to any of the lower animals. Claims have been made of success in this direction, but the fact that all the species of animals used in the experiments are naturally infested with other species of *Chilomastix* and that sufficient precautions were not taken to eliminate such infestations render the reports doubtful and untrustworthy.

**Relation to Disease.**—The evidence connecting this flagellate with disease conditions is purely circumstantial, the principal proof offered by those who believe that it is pathogenic being its frequent occurrence in patients suffering from diarrhoea or dysentery, but it also occurs in perfectly healthy individuals. No lesion of the intestine referable to its presence has been described, and the fact that a careful examination of the stools in the vast majority of patients in whom it occurs, and presenting symptoms of diarrhoea or dysentery, demonstrates the coincident presence of *Endamæba histolytica*, or cultures show the presence of one of the dysentery bacilli, is conclusive evidence, in my opinion, that this flagellate has little or nothing to do with the production of the clinical symptoms. As in the case of the other intestinal flagellates, it is very doubtful if *Chilomastix mesnili* is capable, of itself, of originating an inflammatory condition of the intestine except, perhaps, in very young children when present in very large numbers. It may aggravate an already existing inflammatory condition through its movements over the inflamed mucous membrane of the gut, but that it is a pathogenic parasite *per se* is not believed by the best authorities.

**Prophylaxis.**—Prophylaxis consists of the prevention of the contamination of food or drink by the cysts of the flagellate. The measures necessary to attain this end are the protection of food supplies from flies, the proper disposal of sewage, and personal hygiene, and have already been mentioned in the discussion of prophylaxis against *Giardia intestinalis*.

#### Genus IV. EMBADOMONAS Mackinnon, 1911.

Synonyms: *Waskia*, Wenyon and O'Connor, 1917.

The genus *Embadomonas* was established by Mackinnon, in 1911, the type species being *Embadomonas agilis*, Mackinnon, 1912. Two representatives of this genus have been described as parasites of man, *Embadomonas intestinalis* and *Embadomonas sinensis*.

#### Species I. EMBADOMONAS INTESTINALIS Wenyon. and O'Connor, 1917.

Synonyms: *Waskia intestinalis*, Wenyon and O'Connor, 1917.

**History and Nomenclature.**—*Embadomonas intestinalis* was first described by Wenyon and O'Connor, in 1917. They found it in the faeces of a patient in Egypt, and regarding it as belonging to a new genus,



named it *Waskia intestinalis*. Chalmers and Pekkola (1918) and Dobell (1921) have shown that this flagellate belongs in the genus *Embadomonas* and the proper name for it is *Embadomonas intestinalis*.

**Morphology.**—I have had no opportunity to study this flagellate, and the description that follows is based upon the descriptions published by Wenyon and O'Connor (1917) and Dobell (1921).

*Embadomonas intestinalis* is one of the smallest flagellates occurring in the intestine of man, measuring from 4.5 to 6 microns in length by 2.5 to 4 microns in breadth. It is oval in shape, the cytoplasm appearing finely granular and vacuolated, and containing a nucleus which, in stained specimens, shows a delicate but distinct *nuclear membrane* and



FIG. 35.—*Embadomonas intestinalis*. Dividing form, flagellate form and cyst. (After Wenyon and O'Connor.)

a very small *karyosome*, situated near the centre of the nucleus. The karyosome may be apparently absent or replaced by a collection of minute chromatin granules. The nucleus is situated at the anterior end of the body of the flagellate, just in front of the mouth, or *cytostome*, which appears as a well-marked cleft in the body near the anterior end. The cytostome is supported around its edges by lips, and within them there is a buccal flagellum, as in *Chilomastix*.

In contact with the nuclear membrane, at the anterior pole of the nucleus, and near the cytostome, are two deeply stained chromatin granules, the *blepharoplasts*, and originating from these are two flagella, one directed anteriorly and one directed posteriorly. The anterior flagellum is long and slender and becomes free at once, while the posterior flagellum is shorter and thicker and partly contained within the cytostome of the flagellate.

The organism is actively motile by means of its flagella, the movement being of a jerky progressive character.

**The Cysts.**—Dobell described the cysts as very small, pear-shaped bodies, measuring from 4.5 to 6 microns in length and, in general, resembling those of *Chilomastix mesnili*. They have a definite cystic membrane and contain one nucleus and a deeply stained looped thread, the remains of the fibrils supporting the margins of the mouth in the vegetative form of flagellate.

**Habitat.**—*Embadomonas intestinalis* inhabits the intestine of man, but it has not been determined whether it infests the small or large intestine, or both.

**Species Occurring in Lower Animals.**—A species of this flagellate,

*E. wenyoni*, has been found in the cæcum of a Brazilian monkey by da Fonseca (1917), and another species, *E. cavia*, has been found in the guinea-pig by Wenyon (1922).

**Cultivation.**—Hogue (1921a) claims to have successfully cultivated this flagellate and found that both motile and cystic forms occurred in the cultures, and Wenyon (1922) has cultivated the species of *Embadomonas* occurring in the guinea-pig.

**Life-history.**—Little is known regarding the life-history of this flagellate beyond the fact that it possesses a motile, vegetative stage of development and forms cysts. In the vegetative stage it reproduces by longitudinal binary division, but what development if any, occurs within the cysts has not been determined.

**Geographical Distribution.**—The geographical distribution of *Embadomonas intestinalis* has not been thoroughly determined. Wenyon and O'Connor found it originally in Egypt, and da Fonseca has studied cases of infection with it in Brazil. Kofoed, Kornhauser, and Plate observed it in liquid, diarrhoeal stools in eight American soldiers who were patients in Debarkation Hospital No. 3, New York City. Four of these patients were overseas soldiers and four were home-service men. From these observations it is evident that the species has a wide geographical distribution, and it is probable that further observations will show that it occurs wherever the other intestinal flagellates are found.

**Incidence of Infection.**—*Embadomonas intestinalis* is a rare species of intestinal flagellate, so rare that in many thousands of examinations I have never been able to satisfy myself that I have ever observed an infection with this species. Kofoed and his co-workers, in the examination of nearly three thousand individuals, encountered this flagellate in only eight individuals, as already mentioned, and no other observations are on record of its having been found in the United States.

**Method of Transmission.**—The cysts of this flagellate are presumably the infective agents, but the exact method of infection is unknown. The contamination of food and drink by the cysts is probably the usual method of transmission.

**Experimental Infection of Lower Animals.**—There are no records of the successful transmission of *Embadomonas intestinalis* to any of the lower animals, but closely related species occur in the monkey and the guinea-pig.

**Relation to Disease.**—This flagellate has been found in patients suffering from diarrhoeal attacks, but there is no proof that it was the cause of the attacks. It is, in all probability, a harmless commensal.

Species II. *EMBADOMONAS SINENSIS* Faust and Wassell, 1921.

This species of *Embadomonas* was described by Faust and Wassell in 1921, who found it in the diarrhoeal stools of nine Chinese patients

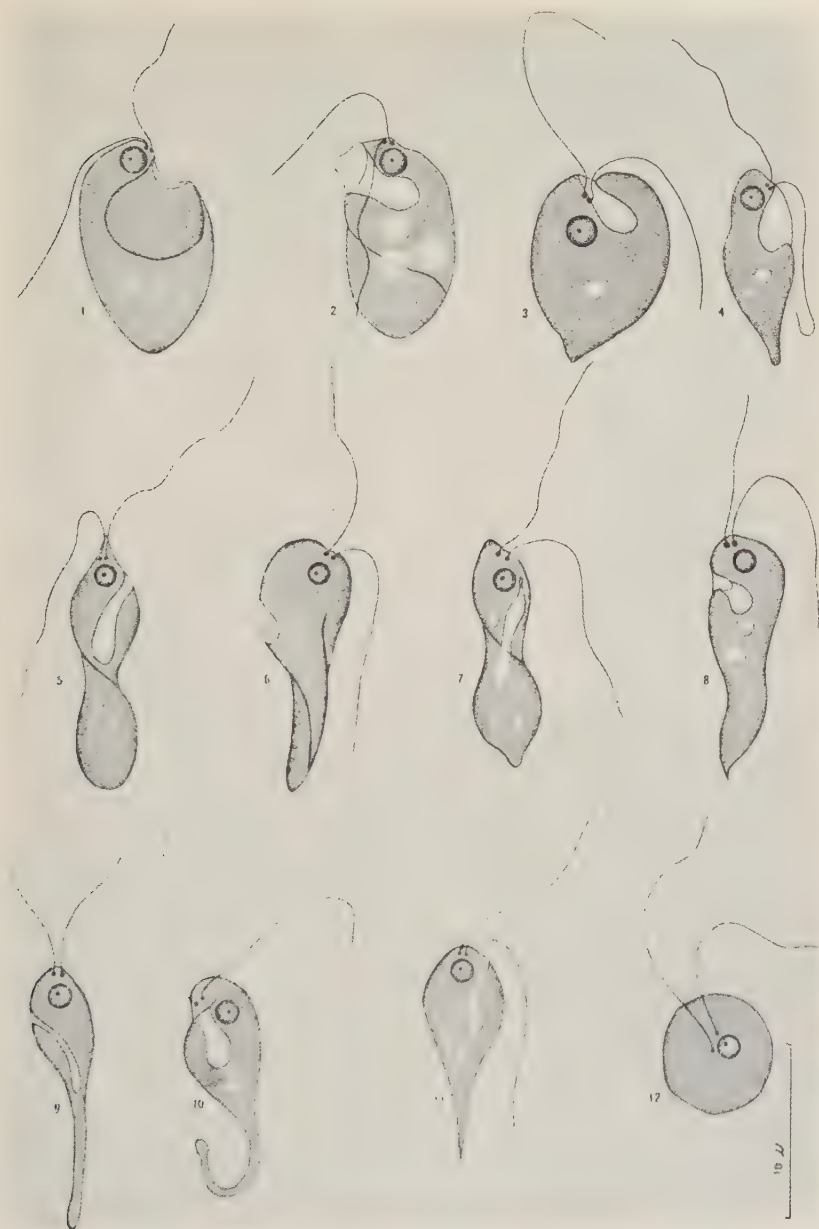


FIG. 36.—*Embadomonas sinensis*. (After Faust.) 1 to 4. Quiescent forms of *Embadomonas sinensis*. 5 to 7. Actively motile individuals. 8 to 11. Elongate individuals. 12. Polar view of quiescent individual.

in the Church General Hospital, at Wuchang, China. It was associated in all but one patient with *Endamæba histolytica*.

**History and Nomenclature.**—Faust (1922) states that the specific characters of *Embadomonas sinensis* agree in general with those of *Embadomonas intestinalis*, but enough differences occur to justify its designation as a new species. These differences consist of the differentiation between the anterior and posterior flagella, the character of the cytostome, and the size of the nucleus.

**Morphology.**—The motile form, or trophozoite, of *Embadomonas sinensis* varies in shape from pyriform to an elongate oval. During active movements, a dimly outlined spiral groove may be observed in some specimens similar to that occurring in *Chilomastix mesnili*. The cytoplasm, in the living specimen, appears finely granular and contains food vacuoles.

The size of the motile form varies, but it averages 14 microns in length by 4.2 microns in breadth, while the quiescent forms had a mean average length of 10 microns and a breadth of 7 microns.

Motile forms were observed with a body length of 20 microns, and Faust and Wassell believe that this species is considerably larger than *Embadomonas intestinalis*.

At the anterior end of the body of the flagellate there are two free flagella, one directed anteriorly, the other backward along the side of the body. There is a large pouch-like mouth or cytostome present in quiescent specimens which becomes much elongated when the body of the organism is attenuated by active movements.

In *stained* specimens a deeply stained granule, or blepharoplast, is observed at the base of each flagellum and at an appreciable distance from the nucleus. The latter is situated at the anterior end of the body, posterior to the blepharoplasts, and is smaller than the nucleus of *Embadomonas intestinalis*. It contains a minute karyosome and has a well-defined nuclear membrane.

**Cysts.**—The cysts are described as small, oval bodies, measuring 6 by 3 microns, and having a well-marked cystic membrane. They are most numerous in the stools after the vegetative forms have disappeared.

**Motility.**—Faust (1922) describes this species as an extremely active flagellate. The movement is progressive in character, consisting of a smooth spiral glide, the turning being so rapid that it can only be seen with difficulty. During movement the body of the flagellate elongates, at which time the dimly outlined spiral groove, already mentioned, may be distinguished. Movement is rendered possible by the synchronous lashing of the two flagella, assisted by the elongation and contraction of the body of the flagellate.



**Habitat.**—The intestine of man. It has not been determined whether this species lives in the large or small intestine or in both.

**Species Occurring in Lower Animals.**—This species has not been found in any of the lower animals, although species of *Embadomonas* do occur in some of the lower animals, as already mentioned in the description of *Embadomonas intestinalis*.

**Cultivation.**—*Embadomonas sinensis* has not been cultivated.

**Geographical Distribution.**—This flagellate has been found only in Chinese at Wuchang, China.

**Incidence of Infection.**—Faust (1922) states that *Embadomonas sinensis* was found in 9 of 57 medical cases at the hospital at Wuchang, or 15.7 per cent., which would indicate that it is a common flagellate in man in the locality in which it was discovered, much more common than *Embadomonas intestinalis* is reported to be where it has been observed.

**Life-history.**—Very little is known regarding the life-history of this species. Faust states that the motile form, or trophozoite, divides by longitudinal fission, with separation of the two daughter flagellates at the posterior end, even before the division of the organelles at the anterior end has been completed. After separation the young flagellates are at first pyriform in shape and quiescent, but soon become motile and elongate. Under certain conditions the trophozoites encyst, but reproduction within the cysts has not been described.

**Method of Transmission.**—The transmission of the infection is presumably through food or drink contaminated by the cysts of the flagellate, but nothing definite is known about it.

**Relation to Disease.**—Faust states that in all but one of his cases the flagellates occurred in association with *Endamæba histolytica*, so that it is impossible to conclude that the diarrhoeal stools in which they occurred were caused by *Embadomonas sinensis* or whether this flagellate is pathogenic. He concludes that it is probably not, of itself, a pathogenic parasite, but that the presence of very large numbers might result in a diarrhoeal condition.

While *Embadomonas sinensis* is accepted tentatively as a distinct species of *Embadomonas*, it is desirable, in view of the close resemblance of this species to *Embadomonas intestinalis*, that more work be done upon this flagellate with reference to the question of its specific position.

## Genus V. ENTEROMONAS da Fonseca, 1915.

Synonyms: *Monocercomonas*, Chatterjee, 1917. *Trichomastix*, Chatterjee, 1917. *Dicercomonas*, Chalmers and Pekkola, 1919.

This genus was established in 1915 by da Fonseca to include a species of flagellate discovered by him in the stools of a patient suffering from dysentery of obscure origin in Brazil. This flagellate he named *Entero-*

*monas hominis*, and this is the only species of this genus that has been described as occurring in man.

Species I. ENTEROMONAS HOMINIS da Fonseca, 1915.

Synonyms: "*Monocercomonas*," Chatterjee, 1917. *Trichomastix hominis*, Chatterjee, 1917. *Dicercomonas soudanensis*, Chalmers and Pekkola, 1919. *Diplocercomonas soudanensis*, Chalmers and Pekkola, 1919. *Enteromonas bengalensis*, Chatterjee, 1919.

**History and Nomenclature.**—The nomenclature of this flagellate is in an unsatisfactory condition and it is practically certain that different observers confused it with other undifferentiated species and with coprozoic flagellates.

The organism was first described by da Fonseca and called by him *Enteromonas hominis*, in 1915. Chalmers and Pekkola (1917) described what they considered an identical flagellate occurring in Egypt, naming it *Dicercomonas soudanensis*, but renamed it *Diplocercomonas soudanensis* in 1919, the generic name *Dicercomonas* not being available. In 1917, Chatterjee described a similar flagellate in India, naming it *Trichomastix hominis*, and in 1919 described a second species which he named *Enteromonas bengalensis*. As the name *Enteromonas hominis* antedates all of the names mentioned, it becomes the proper name for this species, and *Enteromonas bengalensis* is, without doubt, identical with da Fonseca's species.

In 1917, Wenyon and O'Connor described a new species of intestinal flagellate occurring in man in Egypt, naming it *Tricercomonas intestinalis*. Dobell (1921) believes that this flagellate is identical with *Enteromonas hominis*, but recent observations upon Wenyon and O'Connor's species, especially the cultivation experiments of Lynch (1922), appear to be sufficient to prove that this is not so, and that *Tricercomonas intestinalis* is a valid species and that, therefore, the name is not a synonym of *Enteromonas hominis*.

**Morphology.**—*Enteromonas hominis* is a small spherical or oval flagellate, measuring from 5 to 6 microns in diameter in the living condition, stained specimens being slightly smaller. It is actively motile, the movement being progressive in character. The flagellate is so small that it is very difficult to distinguish the number of flagella and their arrangement, but da Fonseca states that there are three free flagella, two directed forward and of equal length, and a shorter one directed backward along the margin of the body, but not attached to the margin. There is no undulatory membrane, cytostome, or axostyle. In the living condition the cytoplasm is finely granular and contains numerous food vacuoles.

In stained preparations a well-defined nucleus is present at the anterior end, near the margin of the organism, which has a definite nuclear

membrane and a large central karyosome. At the anterior pole of the nucleus there is a rhizoplast which connects it with three deeply stained granules or blepharoplasts, from which arise the three flagella. Da Fonseca states that rarely larger forms occur which have more than three flagella irregularly situated, but that these are really degenerated forms in which the flagella have been split and dissociated. The cytoplasm is finely granular, containing food vacuoles which may be filled with bacteria.

No cysts of this species have been described. The cysts described by Wenyon and O'Connor (1917), and which Dobell regards as the cysts of this species, are, I believe, rightly regarded by most authorities as those of *Tricercomonas intestinalis*, as originally described by Wenyon and O'Connor.

**Habitat.**—The intestine of man. What portion of the intestinal tract is preferred by the flagellate is not known.

**Species Occurring in Lower Animals.**—Da Fonseca (1918) reports a species of *Enteromonas*, *E. intestinalis*, occurring in rabbits in Brazil, and Lynch (1922), a species occurring in the guinea-pig, *E. caviae*. Both are distinct from the species occurring in man.

**Life-history.**—Little is known regarding the life-history of this flagellate. Da Fonseca states that it reproduces by binary longitudinal fission, the blepharoplasts and nucleus first dividing, followed by the division of the body and the flagella.

**Geographical Distribution.**—*Enteromonas hominis* has been found in man in Brazil, Egypt, and French Guiana. Hegner and Becker (1922) state that they found flagellates in the stools of two patients in Baltimore, Maryland, that resembled the descriptions of this species, and it is probable that further research will show that it is a widely distributed species, although a rare one.

**Incidence of Infection.**—*Enteromonas hominis* is evidently a rare intestinal flagellate, only a few cases of infection with it being recorded in the literature. However, the ease with which it may be confused with other flagellates may account for its seeming rarity.

**Method of Transmission.**—Unknown, but probably through food and drink, contaminated by the vegetative form of the flagellate, or if cysts are formed, by the cysts.

**Experimental Infection of Lower Animals.**—There are no records in the literature of the experimental infection of any of the lower animals with *Enteromonas hominis*.

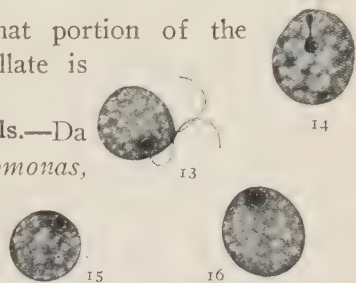


FIG. 37.—*Enteromonas hominis*. (After da Fonseca.) Iron-haematoxylin stain. 13. Organism with flagella in natural position. 14. Flagellate showing the structure of the nucleus and origin of flagella. 15 and 16. Organisms without flagella and showing primitive type of nucleus with large karyosomes.

**Relation to Disease.**—Da Fonseca found this flagellate in the stools from a patient suffering from dysentery of unknown origin in Rio de Janeiro, Brazil, but beyond its association with the symptoms that were present there is no evidence that it was the cause of the disease. At present it is not believed that this flagellate is a pathogenic parasite of man.

Genus VI. *TRICERCOMONAS* Wenyon and O'Connor, 1917.

The genus *Tricercomonas* was founded by Wenyon and O'Connor, in 1917, to include a flagellate which they found in the fæces of a patient in Egypt. Only one species of this genus has been described, *Tricercomonas intestinalis*.

Species I. *TRICERCOMONAS INTESTINALIS* Wenyon and O'Connor, 1917.

**History and Nomenclature.**—In 1917, Wenyon and O'Connor described a new species of flagellate which they discovered during the examination of the stools of soldiers in Egypt. They called this organism *Tricercomonas intestinalis* and their observations have been confirmed by Lynch (1922), who found the same flagellate in the stools of a woman in Texas.

In the opinion of Dobell (1921) *Tricercomonas intestinalis* is identical with *Enteromonas hominis*, but in view of the fact that the two organisms differ in their morphology, as described by their discoverers, who are all trained protozoologists, I believe that the two species are distinct. Even Dobell states that he concludes that the two are identical "with some hesitancy" and believes it possible that they may be distinct. Until more definite proof exists that they are identical, it would seem best to regard *Enteromonas hominis* and *Tricercomonas intestinalis* as distinct species.

**Morphology.**—Wenyon and O'Connor (1917) and Lynch (1922) have described this flagellate and the following description is compiled from theirs, as I have not studied this species personally.

The motile, vegetative forms of the organism are round or oval in shape and measure from 4 to 8 microns in diameter. They are very actively motile, swimming about in a jerky manner, and frequently appear to rotate while progressing. In the active flagellate it is impossible to count the number of flagella, but in stained specimens four flagella are seen, three free and directed anteriorly and one attached for about three-quarters of its length to the body, and directed posteriorly. The flagella apparently originate from blepharoplasts at the anterior end of the body and are so closely associated that they appear to merge into a single small mass.

The nucleus, only visible in stained specimens, is oval in shape and situated at the anterior extremity of the body near the origin of the



flagella. It has a well-defined central karyosome and a definite nuclear membrane. During multiplication the chromatin of the karyosome becomes distributed throughout the nucleus. In cultures Lynch states that he has found forms containing four nuclei, indicating multiple division of the flagellate. The cytoplasm is finely granular and contains small food vacuoles.

**Cysts.**—The cysts of *Tricercomonas intestinalis* have been described by Wenyon and O'Connor (1917) and Lynch (1922). They are oval in shape and have a well-defined cystic membrane. They measure from 4 to 8 microns in length and from 3 to 4 microns in breadth. From one to four minute nuclei are observed within the cysts, the fully developed

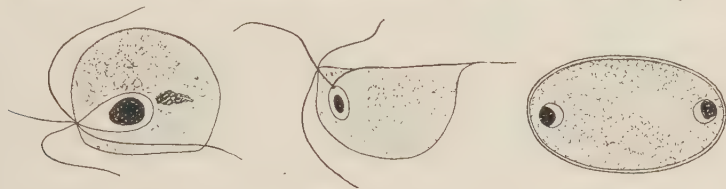


FIG. 38.—*Tricercomonas intestinalis*. Two flagellate forms and one cyst. (After Wenyon and O'Connor. Pub. Wellcome Bureau Scientific Research.)

cyst containing four nuclei. The nuclei are situated at the poles of the cyst, those most frequently observed containing a nucleus at each pole. Very deeply stained chromidial bodies may sometimes be observed within the cysts.

**Habitat.**—Lynch (1922) states that this species of flagellate inhabits the large intestine, as it was not found, in his case, in material from a duodenal drainage. However, it cannot be said that the exact habitat of *Tricercomonas intestinalis* within the intestine has been ascertained. It has only been found in the intestine of man.

**Species Occurring in Lower Animals.**—No species of this genus have been reported as occurring in any of the lower animals.

**Cultivation.**—Lynch (1922) reports the successful cultivation of *Tricercomonas intestinalis* in ascitic fluid to which had been added four parts of 0.9 per cent. salt solution. No cysts were observed in the cultures and all the flagellates died within four days, transplants not being successful. Apparently the most that Lynch accomplished was to keep the flagellates alive for a few days outside the human body.

**Life-history.**—This flagellate has a motile, vegetative stage of development and a cystic stage. In the former, reproduction occurs by binary longitudinal division, and in cultures Lynch found forms containing four nuclei indicating multiple division. In the cysts four daughter flagellates are developed.

The details of multiplication, either in the vegetative or cystic forms, have not been clearly worked out. Lynch states that in the vegetative

form the nucleus divides into two, followed by the division of the flagella, before the division of the body occurs.

**Geographical Distribution.**—This flagellate has been found in Egypt and in the United States. Further research will probably show that it has a world-wide distribution.

**Incidence of Infection.**—There are not sufficient data of record in this respect upon which to base any statement as to the incidence of infection with *Tricercomonas intestinalis*. The indications at present are that it is a rare species of intestinal flagellate.

**Method of Transmission.**—The exact method of transmission is unknown, but it is probably through food and drink contaminated by the cysts of the flagellate.

**Experimental Infection of Lower Animals.**—There is no instance of the experimental infection of any of the lower animals with this flagellate.

**Relation to Disease.**—At the present time there is no evidence that *Tricercomonas intestinalis* is other than a harmless commensal in the intestine of man.

## Genus VII. CRAIGIA Calkins, 1912.

This genus was established by Calkins, in 1912, to include a parasitic organism of the human intestine which I described in 1906, and named *Paramæba hominis*. This organism was believed to be an amœba, and the genus *Craigia* was established by Calkins as a genus of amœbæ, but Kofoid and Swezy (1921) have shown that this parasite is probably a flagellate, and have adopted Calkins' generic name *Craigia* as the name of a genus of flagellates to which this organism belongs, in their opinion.

### Species I. CRAIGIA HOMINIS (Craig, 1906) Calkins, 1912.

Synonyms: *Paramæba hominis*, Craig, 1906.

In 1906, I described a protozoan parasite occurring in the stools of patients suffering from diarrhœa observed at Manila, P. I., which I considered a new species, and because of its resemblance to *Paramæba eilhardi*, Schaudinn, 1896, I placed it in the genus *Paramæba* and named it *Paramæba hominis*. In 1912, Calkins called attention to the fact that this parasite had only one flagellum in the flagellate stage, while *Paramæba eilhardi*, as described by Schaudinn, had two flagella, and that the accessory nuclear body of *Paramæba hominis* was probably not identical with that of *Paramæba eilhardi*. For these reasons he believed that it could not be considered as belonging to the genus *Paramæba*, and created a new genus, *Craigia*, to contain it, naming the parasite *Craigia hominis*.

In 1921, Kofoid and Swezy transferred *Craigia hominis* to the MASTIGOPHORA (FLAGELLATA) on the ground that the accessory nuclear

body is a blepharoplast, a structure characteristic of flagellates, and that during mitosis a paradesmose is formed between the dividing centrosomes and the nuclear membrane, the paradesmose also being a structure characteristic of the flagellates. I accept Kofoid and Swezy's interpretation of the systematic position of this parasite, and now consider it an intestinal flagellate of man.

Hartmann (1912), Dobell (1919), and Brumpt (1922) believe that *Craigia hominis* is not a specific organism, but was described from a mixture of amœbæ and flagellates. This is merely an opinion on the part of these observers and merits little attention in view of the confirmation of this species by Barlow (1915), Tyau (1917), and Kofoid and Swezy (1921). The latter observers have studied cases of infection with this parasite and state (1921), "One of the most important flagellate infections of the human intestine appears to be that of *Craigia*, an organism first described as *Paramœba*, with flagellate, amœboid, and encysted stages," and among the scientific results obtained by Kofoid at the University of California, listed in the Carnegie Institution Year-book, No. 21, 1922, is "The establishment of craigiasis as a human disease with an etiologial factor, *Craigia hominis*. This has been denied by English investigators without adequate data."

In a personal letter, dated June 7, 1923, Professor Kofoid writes: "My observations have led me to conclude that these organisms (*Craigia*) represent a distinct parasitic and probably pathogenic entity, that they are not a mixture of amœbæ and flagellates, and that they are not coprozoic, since they occur in warm stools in both flagellated and encysted stages."

In view of these observations of Kofoid, Kofoid and Swezy, and my own, I believe that *Craigia hominis* is a valid species, and that the opinions of those who have never studied the parasite, but who deny its existence, should receive little credence in view of the positive evidence furnished by our observations.

A second species of *Craigia* was described by Barlow, in 1915, and named by him, *Craigia migrans*. Kofoid and Swezy (1921) have studied infections with this parasite and state that further evidence is required before the independence of the two forms can be considered as established.

**Morphology.**—*Craigia hominis* possesses both an amœbic and flagellate stage of existence, reproducing during the amœbic stage by simple division for a number of generations, at the end of which time it encysts, and within the cyst there develop numerous small swimmers which finally escape, develop a single flagellum, and reproduce by longitudinal division for several generations, after which the flagellum disappears, the organism develops amœboid motion, and again begins the amœbic stage of development.

For convenience of description the morphology in the amœbic stage, the cystic stage, and the flagellate stage of development will be considered separately.

**Morphology in Amœbic Stage.**—The *size* of the living organism at this stage of development varies considerably, the diameter of the resting amœba varying from 10 to 25 microns, the average measurement being about 15 to 18 microns. The *shape*, when motionless, is round or oval, but when moving it is very irregular and several pseudopodia may be extruded from the periphery of the organism at the same time. *Motility* in fresh preparations is very active, progressive motion well marked, and there is a clear distinction between the ectoplasm and the endoplasm, the latter being much more refractive, the ectoplasm being thin and veil-like in consistence. In the motionless amœba there is no distinction between the ectoplasm and the endoplasm, and in the smaller amœbæ this distinction is frequently lost during motion. The *ectoplasm*, which forms the pseudopodia, is homogeneous in structure, and the pseudopodia vary in shape, usually being rather broad and conical in appearance, but in the fully developed amœbæ they may be slender and almost pointed. The endoplasm is reticular in structure, often filled with food vacuoles, which may contain bacteria, crystals, and, in rare instances, red blood corpuscles.

In the living condition a *nucleus* can almost always be distinguished, appearing as a refractile, spherical body having a thick and more refractive nuclear membrane, often appearing to be composed of refractile rods arranged end to end around the periphery of the nuclear substance. In some of the fully grown amœbic forms a brightly glistening, minute oval body may be observed lying near or in contact with the nucleus, the blepharoplast.

In *stained* specimens the ectoplasm and endoplasm are not differentiated, the cytoplasm appearing reticulated and containing small vacuoles, bacteria, crystals, and red blood corpuscles, in some instances. With the hæmatoxylin stains the nucleus stains well, the nuclear membrane appearing as a rather thick black ring upon the inner side of which there may be located isolated granules of chromatin. In some instances this chromatin appears to form a rather uniform layer lining the inner surface of the membrane. The karyosome is large and situated at the centre of the nucleus. It stains uniformly and does not contain a centriole. In very well differentiated specimens the karyosome is seen to be composed of chromatin granules closely packed together. The space between the karyosome and the nuclear membrane is free from chromatin granules and appears as an unstained halo surrounding the karyosome.

The blepharoplast is deeply stained and appears as a deep black dot or a thick, oval rod, lying near or almost in contact with the nucleus. Dividing



forms are sometimes observed in stained specimens, and these will be considered in the discussion of the method of reproduction of the organism.

**Morphology of the Cystic Stage.**—The size of the cysts of *Craigia hominis* varies from 15 to 20 microns, the average cysts measuring about 15 microns in diameter. There is a well-marked pre-cystic stage in which the amœbæ become motionless, contract slightly, and expel all

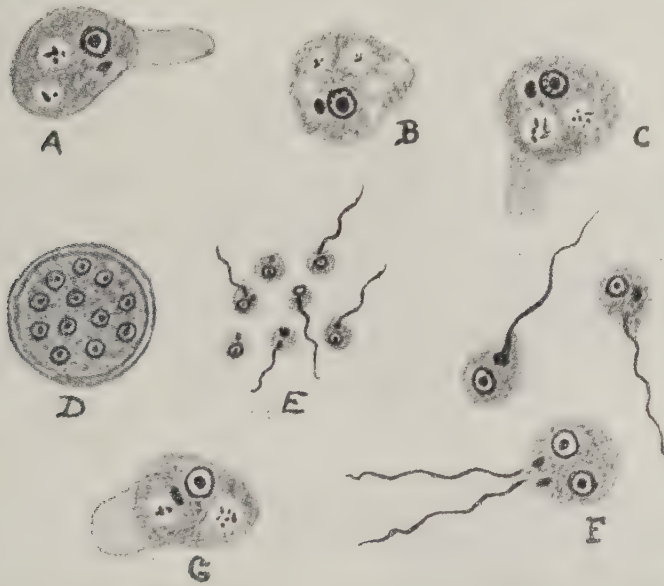


FIG. 39.—*Craigia hominis*. Amœboid and flagellate stages of *Craigia hominis*.  $\times 1,500$ . D.  $\times 1,800$ . A, B, C. Typical amœboid forms showing the character of the nucleus, the blepharoplast, the distinction between the ectoplasm and the endoplasm in the pseudopodium, and the presence of food vacuoles containing bacteria. D. Cyst containing many nuclear bodies which represent the swimmers or young flagellate forms. E. Young flagellate forms showing nucleus, blepharoplast and single flagellum. F. Two fully developed flagellate forms and a dividing flagellate form. In one the flagellum definitely arises from the blepharoplast while in the others there is a considerable space between the blepharoplast and the origin of the flagellum. In the dividing form the nucleus, blepharoplast and flagellum have divided, but the body of the parasite has not divided. G. Amœboid form which has developed from the flagellate form. Note nucleus, blepharoplast, food vacuoles and pseudopodium.

ingested material. A peculiar process of rotation now occurs during which the organism rotates rapidly upon its axis for some time. When rotation ceases a definite cyst wall, having a double outline, is present, but I do not know that rotation is necessary for the formation of the cyst wall. When the cyst wall is present the cysts appear as refractive, colorless, circular bodies, containing a refractile nucleus and blepharoplast. The older cysts do not show a definite nucleus or blepharoplast, but appear to be filled with refractile spherical bodies, the swimmers.

The cysts stain very intensely with the hæmatoxylin stains apparently because of the richness in chromatin, but in very well differentiated spec-

imens one may observe numerous small ring-like bodies which may represent the nuclei of the swarmers.

**Morphology of the Flagellate Stage.**—I have never observed the emergence of the swarmers from the cysts of *Craigia hominis*, but groups of these organisms may be observed arranged in a spherical mass and surrounded by granular detritus, evidently the remains of the cyst.

The young flagellates appear at first as almost spherical bodies, measuring from 3 to 6 microns in diameter, having a finely granular cytoplasm and a poorly defined refractile nucleus. Some of the young forms have a single, very delicate flagellum which enables the organism to move in a jerky manner. The flagellum is about three times the length of the body of the parasite and projects anteriorly, but motion may occur either forward or backward, although usually motion is observed in the direction of the flagellum, which is anterior.

When fully developed the flagellates measure from 10 to 20 microns in length, but are almost circular in shape, being slightly elongated at the portion from which the flagellum arises. The cytoplasm appears finely granular and the nucleus is generally visible as a round, refractile body situated at or near the centre of the organism and having a delicate, but fairly well defined, refractile nuclear membrane. In some of the flagellates the blepharoplast may be distinguished as a brightly refractile dot or rod near the origin of the flagellum or near the nucleus.

In *stained* specimens the cytoplasm stains rather intensely, and it is often difficult to make out the structure of the nucleus. The *nuclear membrane* is thinner than in the amœbic stage and is composed apparently of minute granules. The *karyosome* is large and deeply stained, centrally located in the nucleus, and surrounded by an unstained area. As a whole, the nucleus in the flagellate stage is very similar to that of the amœbic stage, and markedly resembles the nucleus of a *Vahlkampfia*, except that it is smaller, as a whole, and the karyosome is smaller.

The *blepharoplast* stains intensely with the hæmatoxylin stains, appearing as a deep-black dot or rod either in the vicinity of the nucleus or near the origin of the flagellum. I believe that the flagellum takes its origin from the blepharoplast, as in other flagellates, but it is often noted that the blepharoplast is at some distance from the point of origin of the flagellum and sometimes almost in contact with the nucleus. In stained specimens the nucleus is generally situated anterior to the centre of the body and sometimes quite near the origin of the flagellum. Kofoed and Swezy (1921) state that a rhizoplast connects the blepharoplast and the nucleus and that the flagellum arises from the blepharoplast.

The flagellum stains poorly because of its extreme delicacy, but when well stained it appears to be about two and a half to three times as long as the longest diameter of the body of the parasite.

Dividing forms of the flagellate stage of *Craigia hominis* are sometimes observed in stained specimens, containing two nuclei, two blepharoplasts, and showing the beginning of the division of the flagellum at its point of origin, while others are observed in which the division of the two flagellates is almost complete, including the flagellum.

*Craigia migrans*, a second species of *Craigia*, described by Barlow, in 1915, also passes through an amœbic, cystic, and flagellate stage in its life-cycle, but Barlow stated that division did not occur in either the amœbic or flagellate forms, but only in the cysts. However, Kofoid and Swezy (1921) state that they observed a *Craigia* which resembled *Craigia migrans* that divides in both flagellate and amœboid phases, and that they are inclined to believe that Barlow's statement upon this point is in error. The amœboid form of *Craigia migrans* measures from 12 to 30 microns in diameter, averaging about 20 microns in diameter; the cysts average about 18 microns in diameter; while the swarmers measure from 3 to 7 microns in diameter, but may measure as much as 8 microns in diameter. The general morphology of the two species is practically identical, and I believe that, in all probability, *Craigia migrans* is identical with *Craigia hominis*, in view of the finding of Kofoid and Swezy that division occurs in both the amœboid and flagellate forms of *Craigia migrans* as in *Craigia hominis*. The differences in morphology that have been noted in the two supposed species might well be the result of fixing and staining, or due to physiological conditions, as suggested by Kofoid and Swezy.

**Differential Diagnosis.**—Owing to the apparent ease with which *Craigia hominis* may be confused with other flagellates or amœbæ present in human fæces, the differential diagnosis of the parasite merits a brief discussion.

In my original description (1906) of the parasite, I called attention to the liability of confusing it with other flagellates and amœbæ, and described some of the differential features that made its distinction possible. At that time, the utmost care was taken to render the chance of mistaking other flagellates or amœbæ for a new species impossible, and both then, and in my later observations, extreme care was taken in the observations to exclude any stages in the life-history of any of the intestinal flagellates or amœbæ from the description of *Craigia hominis*. Other writers, who have never seen the parasite, may believe that such confusion occurred, and that this parasite was described from a mixture of flagellates and amœbæ, but that such a mistake occurred is impossible, as proven by the confirmation of *Craigia hominis* by others, especially by the recent researches of Kofoid and Swezy, who have studied cases of infection with this parasite and have placed the organism among the flagellates.

*Craigia hominis* is distinguished from any of the parasitic amœbæ of the human intestine, during its amœboid stage, by the character of the nucleus, the presence of a blepharoplast, and the production within the cysts of numerous swarmers; from the intestinal flagellates, during its flagellate phase, it is distinguished by its size, the presence of only one flagellum, and the almost circular shape of the organism. Its cysts could by no means be confused with cysts of *Giardia intestinalis*, or *Chilomastix mesnili*, as they differ so markedly in structure. The amœboid forms of *Trichomonas hominis* which are sometimes observed are distinguished from *Craigia hominis*, in the amœboid phase, by the smaller size, the presence of an undulating membrane, the lack of progressive amœboid motion, and the presence of flagella. In degenerating *Trichomonas hominis* the flagella may have disappeared, and there may be a very limited form of amœboid motility, but such forms could not be confused with the motile forms of *Craigia hominis*, as there is not even a superficial resemblance between them. In fact, it is almost impossible to conceive, in my opinion, how any of the phases of the life-history of *Craigia hominis* could be confused with other intestinal parasites of man except by one having absolutely no experience in the study of these parasites. The flagellate form is easily recognized by its single flagellum arising apparently from the blepharoplast, and by the large central karyosome of the nucleus. The amœboid form is recognized by its characteristic nucleus and by the presence of a blepharoplast, in many of the organisms, while the cysts are recognized, in the early stages, by the presence of the characteristic nucleus and blepharoplast, and the fully developed cyst by the presence of numerous bodies within it, the swarmers. Attention to the morphological characteristics of each of the forms of *Craigia hominis* described should render the confusion of this parasite with the other flagellates and the parasitic amœbæ of the human intestine impossible. Free-living amœbæ of the *Vahlkampfia* type might be confused with the amœboid phase of *Craigia hominis*, but such free-living amœbæ never occur in such large numbers in the stools as does the amœboid form of *Craigia hominis*, as pointed out by Kofoed and Swezy (1921).

The amœbic, cystic, and flagellate phases of *Craigia hominis* may occur simultaneously in the freshly voided stools, but when acute diarrhœal symptoms are present the flagellate forms are generally most numerous. In the chronic cases, in which diarrhœa is intermittent, and the stools are semi-formed or formed, the amœboid and encysted forms are usually most numerous, although flagellate forms may often be observed. Active amœboid forms are rare in formed stools, but in semi-formed stools such organisms are numerous at times. After a saline cathartic all three forms of the parasite can usually be observed, the



flagellate and amœboid forms being most numerous. Kofoed and Swezy (1921) call attention to the fact that in chronic cases the occurrence of any form of *Craigia hominis* in the stools is very erratic and, in my experience, days may pass without any organisms appearing in the stools, only to be followed by a reappearance of the parasite in large numbers and generally in the flagellate and amœboid stages of development.

**Habitat.**—*Craigia hominis* is a parasite of the human intestine, but just what portion of the intestinal tract is its natural habitat has not been determined. The fact that the amœboid, cystic, and flagellate phases all occur in freshly voided stools indicates that the parasite probably lives within the large intestine, and it is probable that it is a parasite of this portion of the intestine.

**Species Occurring in Lower Animals.**—So far as known, *Craigia hominis* does not occur in any of the lower animals. Two somewhat similar organisms have been described as occurring in species of marine worms, but it is very doubtful if they are co-generic with *Craigia hominis*.

**Cultivation.**—The cultivation of this parasite has not been accomplished, all efforts in this direction having met with failure. Kofoed and Swezy (1921) report negative results in attempts to cultivate it in alkaline or neutral peptone water or in ascitic fluid.

**Life-history.**—As stated, *Craigia hominis* passes through an amœboid, cystic, and flagellate phase in its life-history, in each of which multiplication occurs. As infection of man is, in all probability, acquired by swallowing the cysts of the parasite in contaminated food or drink, it is probable that the first phase in its life-cycle is the flagellate phase, the cysts, containing the swimmers, excysting in the intestine, and liberating them after which the typical flagellate develops. After multiplying for an unknown period as flagellates, the flagellum is lost and the amœboid phase begins. During this phase multiplication also occurs, resulting in the formation of the same type of organism, and eventually the cysts are produced which contain the swimmers and which are the infective stage of the parasite.

The above conception of the life-cycle of *Craigia hominis* is theoretical, as we have no definite knowledge beyond the occurrence of the various forms, and the fact that multiplication forms are observed during each phase of the life-cycle.

**Method of Reproduction.**—During the amœboid phase the nucleus divides by mitosis and well-marked mitotic figures are frequently observed. The blepharoplast first divides, as evidenced by the occurrence of amœboid forms containing two blepharoplasts and but one nucleus, which generally presents evidences of mitosis. During division the chromatin of the karyosome splits up into definite masses, the nucleus becomes spindle-shaped, polar caps are formed, connected by delicate strands of

chromatic material, and frequently a well-marked equatorial plate is present, and two distinct rows of granules may be distinguished stretching across the nucleus. In many of the amœboid forms the elongated nucleus presents cup-shaped masses of chromatin at the poles of the nucleus, while the intervening space is filled with chromatin granules and delicate threads of chromatic material without any very definite arrangement. Kofoid and Swezy (1921) state that a paradesmose is formed between the dividing centrosomes and the nuclear membrane. After the division of the nucleus is completed the body of the parasite divides into two and in the young organisms the structure of the nucleus is like that observed in the large amœboid forms.

Owing to the diffuse staining of the cysts I have been unable to follow the method of reproduction within the cysts. In the newly formed cyst the nucleus and blepharoplast are distinct, but in the fully developed cyst they have disappeared, and are replaced by numerous minute, circular bodies, evidently the nuclei of the swarmers.

**Geographical Distribution.**—Cases of infection with *Craigia hominis* have been observed in the Philippine Islands (Craig, 1906), in Honduras (Barlow, 1915), in China (Tyau, 1917), in the United States (Craig, 1911, Kofoid and Swezy, 1921, and Barlow, 1915), in a native of Alaska (Kofoid and Swezy, 1921), and in a missionary from India (Kofoid and Swezy, 1921). In view of these findings, it is evident that the parasite has a wide geographical distribution, and it is probable that further research will show that it has a world-wide distribution.

**Incidence of Infection.**—Practically nothing is known as to the actual incidence of infection with *Craigia hominis*, but it is very evident that it is a rare parasite. In the many thousands of stool examinations that I have made I have found this parasite in only 10 individuals, 6 in the Philippine Islands and 4 in the United States, and all in soldiers of the United States Army. This data covers the period between 1906 and 1914, and since 1914 I have had little opportunity of making such examinations. Kofoid and Swezy (1921) report 6 cases of infection with *Craigia*, all but one of which showed organisms resembling *Craigia migrans* rather than *Craigia hominis*, but in one case, a missionary from India, there was a heavy infection with *Craigia hominis*.

Barber (1915) observed 5 cases of infection with *Craigia hominis* in Honduras, and 51 cases of infection with *Craigia migrans*.

**Method of Transmission.**—Nothing is definitely known regarding the method of transmission of this parasite, but it is almost certain that it is transmitted to man by his swallowing the cysts present in contaminated food or drink.

**Experimental Infection of Lower Animals.**—Efforts to infect kittens

with *Craigia hominis* resulted negatively. So far as known no experiments have been undertaken with other animals.

**Relation to Disease.**—All of the cases of infection with this parasite that I have observed were in hospital patients suffering from a form of chronic diarrhœa of unknown etiology. There was no evidence that this parasite was the cause of the condition beyond the fact of its presence and that treatment resulting in the disappearance of the symptoms also resulted in the disappearance of the parasite.

If *Craigia migrans* is identical with *Craigia hominis*, as I believe, this parasite is apparently the cause of a form of chronic diarrhœa and dysentery occurring in Honduras, as Barlow observed 51 cases of diarrhœa and dysentery there in which *Craigia migrans* was present and in which treatment with emetin resulted in the disappearance of the parasite and the clinical symptoms.

Kofoed and Swezy (1921) found *Craigia hominis* in cases of chronic diarrhœa and regard it as probably a pathogenic parasite. At the present time it may be stated that the evidence available is in favor of *Craigia hominis* being a pathogenic flagellate, causing a form of chronic diarrhœa or dysentery in man.

**Prophylaxis.**—As infection of man with this parasite undoubtedly occurs by swallowing the cysts in contaminated food or drink, what has been said regarding the prophylaxis of *Endamœba histolytica* is equally applicable to the prophylaxis of this flagellate.

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## CHAPTER VII

### INTESTINAL FLAGELLATES OF UNCERTAIN OR DOUBTFUL STATUS. COPROZOIC FLAGELLATES. FLAGELLATES OF THE VAGINA AND MOUTH. DIAGNOSIS.

**Intestinal Flagellates of Uncertain or Doubtful Status.**—The literature relating to the intestinal flagellates is filled with descriptions of flagellated organisms occurring in the intestine of man which are so incomplete or unsatisfactory that it is impossible to classify the organisms to which they refer with any scientific accuracy. Many of these flagellates described under new names are undoubtedly identical with well-known species, while others are obviously coprozoic in origin, having contaminated the fæces after passage from the human body. It would be useless repetition to describe most of these so-called species, but a brief description follows of species that may possibly merit true specific rank, although they need further study and confirmation.

#### Species I. TETRACHILOMASTIX INTESTINALIS, Sangiorgi, 1917.

This flagellate was found in the fæces of an Italian soldier, a patient in the Military Hospital at Venice, by Sangiorgi (1917).

*Tetrachilomastix intestinalis* is described as a pyriform organism, the degenerating forms frequently appearing spherical or elongated in shape. It is actively motile and has four anterior flagella arising from two blepharoplasts situated at the anterior end of the body. The flagella are of equal length. There is an oval or round nucleus near the anterior end of the body which contains a small karyosome. Reproduction occurs by binary longitudinal division and small spherical cysts are formed.

Sangiorgi claims to have cultivated this flagellate from the fæces in peptone water, carrying the cultures along for two months. This fact is very significant of its possible coprozoic origin and his results remain to be confirmed.

#### Species II. TETRACHILOMASTIX BENGALENSIS, Chatterjee, 1923.

This flagellate is said by Chatterjee to be very common in India in the stools of patients suffering from chronic intestinal complaints.

*Tetrachilomastix bengalensis* is described as an elongated pyriform parasite, actively motile, having an undulating membrane, nucleus, cytostome, and four flagella. The nucleus is ring-shaped with a large karyosome. Two pairs of basal granules are situated in front of the

nucleus. The nucleus is situated at the edge of the cytostome. There are four flagella projecting anteriorly and arising near the nucleus. The undulating membrane arises anteriorly near the origin of the flagella and extends backward, crossing the cytostome, and ends near the posterior end in a free flagellum. The anterior end of the parasite is rounded, while the posterior is elongated and pointed.

Chatterjee also described small oval parasites and pre-cystic and cystic stages of the organism. His description of the cysts is unsatisfactory, although he stated that in many of them the nucleus, the cytostome, and the undulating membrane can be seen.

The length of the large flagellated forms varies from 16 to 36 microns and the breadth from 6 to 11 microns. The small, oval forms are 5 to 6 microns in length and from 2 to 3 microns in breadth. The measurements of the pre-cystic and cystic forms are not given.

It is more than probable that this species is identical with *Chilomastix mesnili*, and that Chatterjee has misinterpreted some of the organelles which he has described as characteristic of the species. As he states, "It is rather strange that this parasite, which is very common in this country, escaped the notice of renowned workers who came from England to study the intestinal parasites in soldiers stationed during the war in Egypt, Mesopotamia, and Macedonia, among whom were thousands of Indians."

### Species III. DITRICHOMASTIX HOMINIS, Chalmers and Pekkola, 1916, *emend.* Kofoed and Swezy, 1921.

This species was described in 1916 by Chalmers and Pekkola as *Octomitus hominis*, but Kofoed and Swezy (1921) state that it cannot be placed in that genus and suggest the new generic name *Ditrichomonas* for the organism. It was found in the stools of a patient observed by Chalmers and Pekkola in Egypt, who was suffering from diarrhoea. Dobell (1921) suggests that it may be identical with dividing forms of *Enteromonas hominis*, and certainly the description of the flagellate makes one very suspicious that dividing forms of some intestinal flagellate were mistaken for a new species.

*Ditrichomastix hominis* is described by Chalmers and Pekkola as a very minute flagellate, measuring 6 by 3 microns, having an ellipsoidal body and eight flagella. Six of the flagella, arranged in two groups of three each, arise from two blepharoplasts at the anterior margin of the body. Originating from the same blepharoplasts are two axostyles which run posteriorly along the body, each terminating in a flagellum which projects posteriorly. The nucleus lies at the anterior end of the body and has a well-defined karyosome situated excentrically. No cysts have been described.

Species IV. PENTATRICHOMONAS ARDIN DELTEILI  
(Derrieu and Raynaud, 1914), Kofoid and Swezy, 1923.

This parasite was first described by Derrieu and Raynaud (1914), under the name *Hexamastix ardin delteili*. They found it in the stools of a patient suffering from a fatal form of diarrhœa originating in Algeria. The same organism was described about the same time by Chatterjee (1915), who named it *Pentatrichomonas bengalensis*, and later (1917), found the same species in the fæces of 35 cases of flagellate diarrhœa observed in Calcutta. In 1917, Wenyon and O'Connor reported the finding of a five-flagellated trichomonad in man in Egypt, and in 1918, Haughwout, in the Philippines, reported a *Pentatrichomonas* in cases of diarrhœa in Manila. Recently (1923), Kofoid and Swezy have contributed a critical study of *Pentatrichomonas ardin delteili* as observed by them in three cases of chronic diarrhœa. As a result of their work they conclude that it is a "trichomonad flagellate of man distinct from *Trichomonas hominis*," basing their belief in its specific nature upon the presence of five anterior flagella, while *Trichomonas hominis* has but four.

The question of the specific nature of trichomonad flagellates having a varying number of anterior flagella has already been discussed and it is believed that, for the present, it is best to consider all the intestinal trichomonads of man so far described as belonging to the one species, *Trichomonas hominis*. However, Kofoid and Swezy have excellent reasons, from a zoological standpoint, for considering this five-flagellated trichomonad as a distinct species, and eventually, *Pentatrichomonas ardin delteili* may be generally accepted, although at present it is generally regarded as identical with *Trichomonas hominis*.

*Pentatrichomonas ardin delteili* measures from 9 to 20 microns in length and from 7 to 14 microns in breadth. In morphology it is quite similar to *Trichomonas hominis*, but has five anterior flagella instead of four. The shape of the organism is pyriform, the posterior end forming a conical tip from which projects the axostyle. The undulating membrane is well marked and terminates in a free flagellum posteriorly. Cysts have not been demonstrated, and Kofoid and Swezy state that "We have never seen the least trace of encystment in either fresh stools or cultures."

*Pentatrichomonas ardin delteili* is phagocytic for red blood cells in both stools and cultures, but this phenomenon is not characteristic as red blood corpuscles have been seen within *Trichomonas hominis* and recorded by several observers.

Kofoid and Swezy (1923) have succeeded in cultivating *Pentatrichomonas ardin delteili* in 10 per cent. serum of the rabbit or guinea-pig in Locke's solution at room temperature for a period of 98 days, during



which time two sub-cultures were made. They state that Wagener, working in their laboratory and using 10 per cent. human blood serum in Locke's solution as a culture medium, and incubating at body temperature,

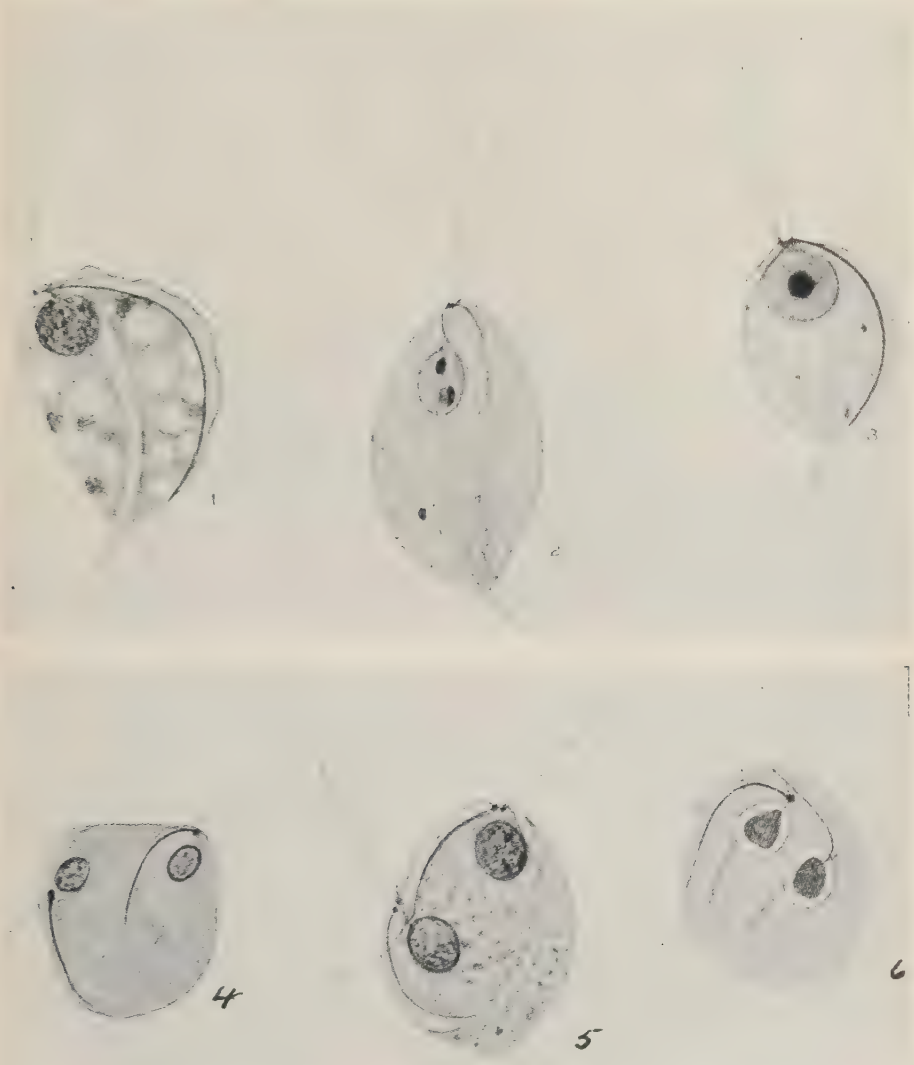


FIG. 40.—*Pentatrichomonas ardin delleili*. (After Kofoed and Swezy.) 1, 2 and 3. Trophozoites showing flagella, undulating membrane, nucleus, blepharoplasts, axostyle and vacuoles. 4, 5 and 6. Dividing forms.

had been able to culture this species for 134 days, with 40 transplants at intervals of about three days. The best cultures were secured in neutral serum, and the organisms died when the pH was 6.2 and 8.2.

Kofoid and Swezy also determined that the organism lived for five days in the stool at room temperature in the laboratory, for three days in water, for 15 days in normal saline solution, and for 118 days in 10 per cent. rabbit serum in Locke's solution.

The pathogenicity of this species has not been determined, but it has generally been observed in cases of chronic diarrhoea.

**Coprozoic Flagellates.**—Certain species of free-living flagellates may be found in the fæces of man and such flagellates are known as coprozoic flagellates. Unable to live and develop in the human intestine, they may pass through it in the encysted form, and thus may be found in the stools immediately after passage, or, if the stools be allowed to stand for some time before examination, the motile vegetative stages of these organisms may be found.

Free-living flagellates are very numerous, living in water and decomposing material, and may be transmitted to man through food or drink contaminated by their cysts, eventually passing out of the body in the fæces, or the fæces may be contaminated after passage, either through the air or by means of water used for cleansing purposes. One of the most common methods by which these coprozoic flagellates reach the fæces is in water used in bed-pans during the collection of the faecal specimens, especially in hospitals.

The fact that the utmost care has been taken in the collection of the fæces for examination does not prevent the occurrence of these flagellates in the fæces, for, as has been stated, they may be swallowed in contaminated food or drink, and, passing through the intestinal canal, be actually voided in the fæces, where they will be found in the encysted stage or, if conditions are favorable, multiplication may occur at once, and the motile forms may be found in the stools a short time after passage.

The coprozoic flagellates are most numerous in the tropics and sub-tropics, and in such regions their occurrence in the fæces of man is exceedingly common. This has led to the greatest confusion in classification and nomenclature of the flagellates, as many of the coprozoic forms have been described, from time to time, as new parasitic species of man, and such mistakes have been made not only by the tyro in protozoology, but by well-trained protozoologists, and even today, there is some difference of opinion regarding the parasitic nature of some of the flagellates that are believed by the majority of zoologists to be coprozoic in character.

In the following brief descriptions of some of the more common coprozoic flagellates I have followed Dobell and O'Connor (1921) and Kofoid and Swezy (1922) as regards classification and general morphology. The species described, in all probability, constitute only a fraction of those that may occur in the fæces, for free-living flagellates abound everywhere in nature, and any of them may, at times, contaminate faecal

material, and most of those described have been claimed, at one time or another, to be parasites of the human intestine.

# 1. CERCOMONAS LONGICAUDA, Dujardin, 1841.

Synonyms: *Cercobodo longicauda* (Dujardin), Senn, 1900. *Cercomonas longicauda* (Dujardin), Wenyon, 1910. *Cercomonas parva*, Hartmann and Chagas, 1910. *Cercomonas longicauda* (Dujardin), Alexeieff, 1911.

This flagellate lives in stagnant water and has been found in cultures from human fæces. The best descriptions of it are those of Wenyon (1910) and Alexeieff (1911).

The organism is pyriform in shape, but the shape varies as amœboid

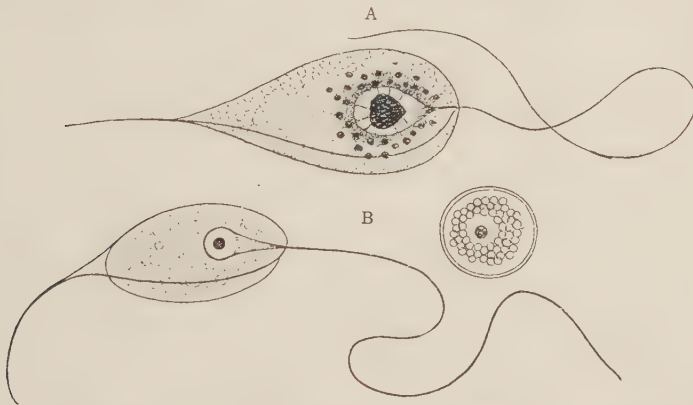


FIG. 41.—*Cercomonas longicauda*. A. Type with granules around the nucleus. (After Wenyon.) B. Type without granules and a cyst. (After Wenyon. Quarterly Journ. Microscop., Science.)

motion is present at certain stages of development. It measures from 5 to 16 microns in length. The cytoplasm is granular and contains food vacuoles filled with bacteria. There is a very long, free anterior flagellum and a shorter posterior flagellum which is adherent to the body of the organism for three-quarters of its length. Each flagellum originates from a tiny blepharoplast situated at the anterior portion of the nucleus, which is located at the anterior portion of the flagellate. The nucleus has a distinct nuclear membrane and a small central karyosome.

The cysts are spherical in shape and measure 4 to 7 microns in diameter. They contain a single nucleus and numerous refractile granules. The cysts are very resistant to drying and are easily carried by air currents to exposed faecal material.

Reproduction in the vegetative stage occurs by simple binary longitudinal division.

*Cercomonas longicauda* can be easily cultivated in hay infusion, peptone water, and upon Musgrave's amœba agar.

## 2. CERCOMONAS CRASSICAUDA, Dujardin, 1841.

This is a common coprozoic flagellate of human faeces and is believed by Woodcock (1916) to be identical with *Cercomonas longicauda*, but this opinion is not shared by recent investigators. Dobell (1921) considers it a distinct species, as does Alexeieff (1911), who has published a very detailed description of the organism.

Its morphology is, in general, similar to that of *Cercomonas longicauda*, but it is a larger flagellate, measuring from 10 to 18 microns in length. It also differs from *Longicauda* in having shorter flagella, the two flagella being little longer than the body. The nucleus has a large karyosome. The cysts measure from 5 to 8 microns in diameter and are spherical in shape. Reproduction in the vegetative form occurs by simple binary longitudinal fission.

## 3. COPROMONAS SUBTILIS, Dobell, 1908.

Synonyms: *Copromonas major*, Berliner, 1909. *Scytomonas pusilla* (Stein), Alexeieff, 1911. *Scytomonas pusilla* (Stein), Schussler, 1917.

*Copromonas subtilis* is an oval flagellate measuring from 7 to 20 microns in length, with an average length of 15 microns. In cultures smaller forms occur, some measuring less than 5 microns in length.

The body is quite rigid, and at the anterior end there is a small mouth, or cytostome. Extending backward from the mouth is a long narrow gullet measuring about half the length of the body. The cytoplasm contains food vacuoles and a single nucleus having a large central karyosome. The space between the nuclear membrane and the karyosome contains chromatin granules and traces of a linin net-work.

A single flagellum, a little longer than the body, arises from a small blepharoplast in the wall of the gullet contiguous to a structure called the reservoir, situated at the anterior end of the flagellate. The reservoir is a clear non-contractile vesicle having at its base a very small contractile vacuole which expels its contents into the reservoir at regular intervals.

The cysts are oval or spherical in shape and measure from 7 to 8 microns in diameter. They have a thin cyst wall and contain a single nucleus.

According to Dobell and O'Connor (1921), reproduction in the vegetative form occurs by binary longitudinal division, fission beginning at the anterior extremity. The blepharoplast first divides into two and a new flagellum grows out of each blepharoplast, the nucleus afterward dividing by a simple form of mitosis.

Dobell (1908) has demonstrated that conjugation occurs in this species, the two conjugants uniting by their anterior ends and gradually



fusing, the nuclei presenting reduction divisions, finally becoming a single zygote nucleus. When conjugation is complete the zygote may become a single large flagellate or encystment may occur, but conjugation is not essential to encystment.

*Copromonas subtilis* is apparently a rare flagellate, so far as its occurrence in human fæces is concerned, as Dobell found it present in only one case, and I have never encountered this organism in thousands of examinations of fæces. A similar species occurs in the fæces of frogs, which Dobell states is indistinguishable from the species he found in human fæces.

Dobell cultivated *Copromonas subtilis* from fæces upon agar plates and was able to study it very carefully in such cultures. It is undoubtedly a coprozoic flagellate of rare occurrence in man.

#### 4. HELKESIMASTIX FÆCICOLA, Woodcock and Lapage, 1915.

This flagellate was first described by Woodcock and Lapage (1915), who found it in the fæces of goats, and it has since been cultivated from a single specimen of human fæces by Dobell (1921). It resembles a *Cercomonas*, but there is only one flagellum, which arises from a blepharoplast at the anterior end. This flagellum is directed posteriorly and is adherent to the surface of the body until it reaches the posterior extremity, when it becomes a free flagellum.

The shape of the organism is oval and there is a pointed anterior extremity which is very rigid. The size varies somewhat, but the average length is from 4 to 6 microns, according to Dobell. Motility is progressive in type, the anterior, pointed end being always in advance.

The cytoplasm is finely granular and alveolar in appearance, and there is a small contractile vacuole, situated either near the centre or in the posterior portion of the body. The nucleus is visible in stained specimens, lying at the anterior end. It is oval or spherical in shape, and has a central karyosome. The single flagellum is apparently attached to the nucleus, the granule from which it arises being situated upon the nuclear membrane.

Reproduction occurs by simple binary longitudinal division. The organism lives upon bacteria which it ingests. No cytostome is present.

Dobell states that he was unable to find cysts in his material, but Woodcock and Lapage described cysts measuring from 3 to 6 microns in diameter. The cysts are spherical in shape and contain a single nucleus.

This flagellate was cultivated by Dobell from the fæces upon agar plates, where it grew very rapidly.

## 5. BODO CAUDATUS (Dujardin, 1841), Stein, 1878.

Synonyms: *Amphimonas caudata*, Dujardin, 1841. *Bodo urinarius*, Hassall, 1859. *Trichomonas irregularis*, Salisbury, 1868. *Diplomastix caudata*, S. Kent, 1881. *Cystomonas urinaria*, Blanchard, 1885. *Bodo asiaticus*, Castellani and Chalmers, 1910. *Prowazekia cruzi*, Hartman and Chagas, 1910. *Prowazekia weinbergi*, Matthis and Leger, 1910. *Prowazekia asiatica* (Castellani and Chalmers), Whitmore, 1911. *Prowazekia javanensis*, Flu, 1912. *Prowazekia urinaria* (Hassall), Sinton, 1912. *Prowazekia italica*, Sangiorgi and Ugdulena, 1916.

*Bodo caudatus* is one of the most common coprozoic flagellates occurring in human fæces and, as indicated by the large number of synonyms, has been rediscovered and redescribed again and again by different observers. It is generally, but, in my opinion, incorrectly, placed in the genus *Prowazekia* by the majority of writers, and I agree with Alexeieff (1911) and Dobell (1921) in regarding the various species of *Prowazekia* mentioned above as synonyms of *Bodo caudatus* as identical with the latter organism.

In the vegetative form the shape varies greatly, being sausage-shaped, carrot-shaped, round, or oval, according to Sinton (1912), but in my experience, the shape is generally roughly oval or leaf-shaped. The size also varies greatly, the length being given by different observers as between 8 and 25 microns and the breadth as from 2.5 to 6 microns, but most of the organisms are from 10 to 15 microns in length. They are actively motile, the motility being of a jerky, progressive character.

The body is usually broadest at the anterior end, the posterior end being pointed, but bluntly oval and spherical forms are sometimes observed, the latter probably being pre-cystic or degenerative in character. The anterior end shows a small lip which projects over the opening of the mouth or cytostome, which is very small and sometimes invisible. The cytoplasm is granular, and numerous food vacuoles are present, especially in the posterior portion of the body. There is a very small contractile vacuole situated near the mouth.

In stained specimens a large nucleus is visible near the middle of the body which has a definite nuclear membrane and a large central karyosome. A deeply stained, oval body, the kintonucleus, or kintoplast, as Dobell prefers to call it, lies at the anterior end of the body near the mouth and in close association with two minute, deeply stained granules, or blepharoplasts, from which arise the flagella.

The flagella are two in number, one directed anteriorly and arising from one of the blepharoplasts, the other, directed posteriorly, arising from the second blepharoplast. The anterior flagellum becomes free at once and is shorter than the posteriorly directed flagellum, which is generally attached for the first part of its length to the body of the flagellate. The flagella originate very near the margin of the mouth or cytostome.

The cysts of *Bodo caudatus* are oval in shape and measure from 5 to 7 microns in length. They contain one nucleus similar in structure to the nucleus of the vegetative form, and a kinetonucleus is also present in the cyst. The cyst may also contain deeply stained granules of chromatin and short filaments representing the remains of the flagella. Division of the nucleus and kinetonucleus may occur in the cysts so that binucleate cysts are sometimes observed.

In the vegetative stage reproduction occurs by binary longitudinal division. In the cystic stage, Sinton (1912) states that a single fully developed flagellate is formed within the cyst and emerges from it under favorable conditions.

*Bodo caudatus* can be easily cultivated in hay infusion, peptone water, urine, diluted blood serum, and upon agar plates. The cultures develop well at 20° C., but die at 37° C.

Hassall found this flagellate in alkaline urine from man which had been passed for several days. It could not be found in freshly passed urine. This fact, and the observation that a temperature of 37° C. is fatal to the cultural forms, is proof that the organism is of coprozoic origin when found in urine or fæces.

Numerous species have been described under the generic name of *Prowazekia*, but these are all identical with *Bodo caudatus*. These species were found in the fæces of man, but were obviously contaminations.

In addition to *Bodo caudatus* another species of *Bodo*, *Bodo edax*, Klebs, 1892, is a common coprozoic flagellate of human fæces. Dobell and O'Connor (1921) state that it may be distinguished from *Bodo caudatus* by its smaller size, more regular oval shape, well-defined cytostome, larger kinetonucleus, and the equal length of the two flagella.

A considerable number of coprozoic flagellates that have been described as occurring in man are not considered here for the reason that the descriptions are so incomplete and unsatisfactory that one cannot classify the organisms with any degree of certainty.

## FLAGELLATES OF THE VAGINA AND MOUTH

A species of *Trichomonas*, *Trichomonas vaginalis*, is sometimes found in the vaginal secretions of women, and another species, *Trichomonas buccalis*, occurs in the human mouth.

### TRICHOMONAS VAGINALIS, Donne, 1837

**History and Nomenclature.**—This species of *Trichomonas* was discovered by Donne (1837) in the vaginal secretions of women, and he established the genus *Trichomonas* to include this flagellate, giving it the specific name *vaginalis*. As this was the first trichomonad described, it becomes the type species of the genus.

*Trichomonas vaginalis* has been described by Kunstler (1884), Dock

(1894), Bensen (1912), and more recently by Reuling (1921), Kofoed and Swezy (1921), and Lynch (1922). All of the recent writers regard it as distinct from *Trichomonas hominis*.

**Morphology.**—*Trichomonas vaginalis* is very polymorphic, being pyriform, fusiform, spheroidal, or amœboid in shape, according to local conditions. The typical flagellate is pear-shaped, having a somewhat rounded anterior end and a pointed posterior extremity.

The size varies, but ranges from 15 to 25 microns in length and 10 to 15 microns in breadth. Smaller forms are observed in cultures measuring from 5 to 10 microns in length. In stained preparations the flagellate is smaller than in the living condition. The organism is actively motile, the motility being progressive in character. Amœboid motion is often observed in the quiescent organisms and is probably a sign of degeneration.

In living specimens the body of the flagellate is colorless, finely granular, and shows numerous food vacuoles. There is a definite undulating membrane running in a spiral manner across the body and four flagella, which can only be counted accurately in the stained specimens.



FIG. 42.—*Trichomonas vaginalis*.  $\times 2,000$ . (After Kunstler.)

In stained preparations the body of *Trichomonas vaginalis* shows an alveolar structure and contains vacuoles which may be filled with bacteria. The nucleus is oval in shape and is situated in the anterior half of the body, generally quite near the anterior end. It contains a large central karyosome. Lynch (1922) states that the nucleus is connected with the origin of the flagella, or blepharoplasts, by a delicate, poorly staining thread, the rhizoplast.

At the anterior end of the body, and often situated in a snout-like projection, is a granular mass, which, in very well stained specimens, may be seen to consist of three or four deeply stained granules, or blepharoplasts. From these arise the flagella, which are typically four in number and of about the same length as the body. The flagella become free at once and are directed anteriorly. They often appear to be joined at their origin and to arise from a single large blepharoplast. Forms of this species are seen having but three flagella. This appearance may be due to degenerative changes, atypical division, or two of the flagella may have become adherent during the staining process.

The undulating membrane arises from the blepharoplastic area and runs backward in a spiral manner, terminating in the body near the posterior end. The undulating membrane in this species does not terminate in



a free posterior flagellum, as it does in *Trichomonas hominis*, and is more narrow and arranged in closer folds.

A poorly stained rod-like structure, the axostyle, arises near the nucleus, in the area of the blepharoplasts, and extends backward through the centre of the body, terminating near the posterior end, where it may project slightly from the surface. The posterior end of the flagellate is pointed and often acutely curved.

The cysts of this species, if they occur, have not been identified.

**Habitat.**—*Trichomonas vaginalis* occurs in the vaginal secretions of women, but is only found when these secretions are acid in reaction. It is not found in the vagina when the secretions are alkaline in reaction even though a diseased condition of the mucous membrane be present. This species has also been found in the urethra of both men and women under certain conditions.

**Species Occurring in Lower Animals.**—No trichomonad has been described as occurring in the genital tract of any of the lower animals, and this species cannot be transmitted to any of the lower animals so far as known.

**Cultivation.**—*Trichomonas vaginalis* has been successfully cultivated from the vaginal secretions by Lynch (1915), (1922) and Reuling (1921). Lynch obtained his first cultures in beef broth, but had greater success later (1922) with liquid human blood serum, diluted with 10 parts of 0.5 per cent. sodium chloride solution. Growth progressed for 4 or 5 days, after which the flagellates gradually die out, but some may remain alive for as long as 12 days. Transplants are successful if made during the first four days. The organisms grow best at the bottom of the tubes and live upon the bacteria in the cultures.

**Life-history.**—Only the vegetative stage of *Trichomonas vaginalis* is known. Reproduction occurs by binary longitudinal fission. Lynch (1922) states that in cultures flagellates of this species are observed showing evidences of mitotic division of the nucleus. After the completion of nuclear division the body splits into two practically equal portions, commencing at the anterior end, there having been a previous division of the blepharoplasts, undulating membrane, and flagella. In this manner two fully developed organisms result from the division.

**Geographical Distribution.**—The geographical distribution of *Trichomonas vaginalis* is probably world-wide. It has been found in Europe, the United States, South America, Japan, and the Philippine Islands.

**Incidence of Infection.**—There are no extensive data of record showing the incidence of infection with this flagellate, but it is comparatively common in women who present abnormal conditions of the vaginal mucous membrane. Brumpt (1913) found it in practically 10 per cent. of women examined in the clinics of Paris.

**Method of Transmission.**—The method of transmission of this flagellate from woman to woman is unknown. In those instances in which *Trichomonas vaginalis* has been found in the urethra and bladder of man, transmission is said to have been through coitus.

**Experimental Infection of Lower Animals.**—There is no record of the successful transmission of *Trichomonas vaginalis* to any of the lower animals. Blochmann (1884) and Dock (1894) endeavored to infect dogs, rabbits, and guinea-pigs, but were unsuccessful.

**Relation to Disease.**—This flagellate occurs only in the vaginal secretions from diseased mucous membranes. It was first observed by Donne in the acid mucus from the vaginal discharges of women, some of whom suffered from venereal disease. It occurs not only in acid mucus, but also in purulent discharges, provided the reaction is acid. If the material becomes alkaline the flagellates disappear, and alkaline vaginal injections will cause their disappearance. The organism may occur in young girls suffering from catarrhal conditions of the vagina when the secretions are acid, and it has been found in the urethra and bladder of women who presented coincidently a vaginal infection with the flagellate. In cases of endometritis, endocervicitis, and vaginitis, the organism is most frequently observed, but even in an apparently normal vagina, provided the reaction is acid, it may be present. The flagellate is often found in the urine in women showing a vaginal infection.

*Trichomonas vaginalis* has been found in the bladder of man by Dock (1894) and in the urine of man by Marchand (1893), Miura (1894), Dock (1894), and others. In all of the cases reported, a coincident pathological condition of some kind was present.

While *Trichomonas vaginalis* occurs only in the secretions and discharges from diseased mucous membranes, there is no adequate evidence available proving that it is the cause of the disease conditions with which it may be associated. The vast majority of investigators believe it to be a harmless commensal, but some are of the opinion that when present in large numbers it may prolong or aggravate any inflammatory condition that may be present at the same time. I believe that in large numbers this flagellate might cause definite symptoms of irritation in an already inflamed mucous membrane, but that it is the cause of the various pathological conditions with which it has been found associated cannot be believed by any careful student of the literature.

TRICHOMONAS BUCCALIS (Goodey, 1917), Kofoid, 1920

Synonyms: *Tetratrichomonas buccalis*, Goodey, 1917. *Tetratrichomonas hominis*, Ohira and Noguchi, 1917.

**History and Nomenclature.**—In 1773, O. F. Müller described a flagellate occurring in the human mouth which he called *Cercaria tenax*, but his description and figures of the flagellate, which he published in 1786,

are so unsatisfactory that it is impossible to identify it with any flagellate that has been described, and the best authorities agree that Müller's organism must be classed as an undeterminable species.

According to Doflein (1911), the flagellates of the human mouth were studied by Steinberg, in 1862, who divided them into three species which he called *Trichomonas elongata*, *Trichomonas caudata*, and *Trichomonas flagellata*, but modern research has not confirmed the work of Steinberg, as only one flagellate occurs in the mouth and that cannot be identified with any of the species he described.

The trichomonad of the mouth was first adequately described by Goodey, in 1917, and named by him *Tetratrichomonas buccalis*. Curiously enough, both Dobell and O'Connor (1921) and Kofoed (1920), as well as other writers, credit Goodey and Wellings with naming the flagellate, in 1917. As a matter of fact, while Goodey's description of it was published as a part of a contribution by both Goodey and Wellings upon *Endamæba gingivalis*, the description of *Tetratrichomonas buccalis* is published at the end of the paper under Goodey's name alone, and he alone suggests the name *Tetratrichomonas buccalis* for it. Hence it is incorrect to write the name *Tetratrichomonas buccalis*, Goodey and Wellings, 1917, the correct name being, if the genus *Tetratrichomonas* was accepted, *Tetratrichomonas buccalis*, Goodey, 1917.

However, as the type species of the genus *Trichomonas* has four flagella, the generic name *Tetratrichomonas*, based upon the fact that *buccalis* has four flagella, is not admissible and, as Kofoed (1920) has pointed out, becomes a synonym of *Trichomonas*, and the proper name of the trichomonad of the mouth is *Trichomonas buccalis*.

**Morphology.**—*Trichomonas buccalis* is normally pear-shaped, but the body is so plastic that the shape constantly changes when the organism is in motion. In the living specimens the cytoplasm is colorless, and finely granular, and presents small food vacuoles most numerous toward the posterior extremity.

The size is given by Goodey (1917) as varying from 7 to 12 microns in length, while Ohira and Noguchi (1917) state that the forms occurring in cultures measure from 10 to 15 microns in length and from 4 to 8 microns in breadth. They observed forms in their cultures measuring as much as 25 microns in length, but these were unusual.

The flagellate is very actively motile, the motion being of a jerky, progressive character. When actively motile it is not possible to determine the number of flagella present, but in specimens in which motility has almost ceased I have been able, by very careful adjustment of illumination, to count from 3 to 4 flagella at the anterior end of the body, 4 being the most frequent number observed. The anterior end of the body is rounded and sometimes presents a slight projection at the

portion from which the flagella arise, while the posterior end is usually pointed. The undulating membrane may be distinguished in the living specimen when motility is not too pronounced as a waving folded membrane along one side of the body.

In stained specimens the cytoplasm of *Trichomonas buccalis* stains poorly and food vacuoles, often filled with bacteria, are scattered through the body, while bacteria may also be seen lying apparently free in the cytoplasm.

The nucleus is oval- or spindle-shaped and lies near the anterior end of the body. Goodey (1917) states that it stains so diffusely that he could not distinguish a karyosome, but in the cultural forms, Ohira and Noguchi (1917) state that a distinct karyosome is present, and their findings agree with my own observations.



FIG. 43.—*Trichomonas buccalis*. (After Goodey.) Free swimming trophozoite showing the four anterior flagella, the undulating membrane and the relations of nucleus, blepharoplast, rhizoplast and axostyle.

Situated in front of the nucleus and at the base of a small projection of the anterior end of the body, there is a deeply stained granular mass, or blepharoplast, connected with the anterior pole of the nucleus by a deeply stained fibril, the rhizoplast. The blepharoplast generally appears as a single granule, but in very finely differentiated specimens it may be seen to be composed of from two to four minute granules in very close association.

The flagella, four in number, arise from the blepharoplast and project anteriorly, becoming free almost at once. They are very delicate and vary slightly in length. In cultural forms two of them are longer than the other two according to Ohira and Noguchi (1917). The flagella usually appear to have a common origin in one blepharoplast, but rarely the blepharoplast is divided into two or more granules and it can then be seen that each flagellum arises from a separate granule.

The undulating membrane is well seen in some stained specimens and arises from a blepharoplast at the anterior end near the origin of the flagella and extends backward along one side of the body for about two-thirds of the body length, when it merges into the body and does not terminate in a free posterior flagellum, as in *Trichomonas hominis*. According to Ohira and Noguchi, the cultural forms sometimes show a short, free flagellum formed by the termination of the undulating membrane, but the forms observed in the mouth do not show this structure.



An axostyle is present, originating near the nucleus and running posteriorly through the body to terminate at the posterior end in a free pointed extremity which projects slightly from the body surface. The axostyle stains well and is rod-like in appearance.

No cysts are known for this species, and cysts do not occur in the cultures of *Trichomonas buccalis*.

**Habitat.**—The flagellate is found in the human mouth in the tartar around the teeth, in the material found in the cavities of carious teeth, and in the pus pockets in cases of pyorrhea alveolaris. Perfectly healthy mouths may harbor the organism, but it occurs more frequently and in greater number in the mouths of individuals presenting some pathological condition of the mucous membrane, or in those who pay little attention to dental hygiene. In cases of Vincent's angina I have found the flagellate in the necrotic tissue in the ulcerated areas in a few cases.

**Species Occurring in Lower Animals.**—No distinct species of *Trichomonas* has been described as present in the mouth of any of the lower animals, and *Trichomonas buccalis* has never been found in any of the lower animals.

**Cultivation.**—*Trichomonas buccalis* was first cultivated by Lynch (1915) and more recently by Ohira and Noguchi (1917). Lynch cultivated this flagellate in neutral and acid beef broth, but Ohira and Noguchi obtained the best results in a mixture of ascitic fluid and Ringer's solution in equal parts, containing a piece of fresh tissue. Growth occurs best at 37° C., but daily transfers are necessary at this temperature to keep the cultures going. At temperatures between 22° and 27° C. multiplication occurs, but growth is much slower and transplants may be made every 48 hours.

**Life-history.**—Only the motile vegetative form of *Trichomonas buccalis* is known. Reproduction occurs by binary longitudinal fission, but in cultures Ohira and Noguchi (1917) describe a process of multiple fission, of "budding" (?), in which from 4 to 8 daughter cells are produced from one organism. I have never observed multiple fission in the forms occurring in the mouth and doubt if it ever occurs.

The flagellate feeds upon the bacteria found in the mouth, and as these are most numerous in diseased conditions, or in individuals who are careless about dental hygiene, it is in such mouths that *Trichomonas buccalis* is generally found.

**Geographical Distribution.**—The geographical distribution of this species is probably world-wide. I have observed it in the mouths of individuals in the Philippine Islands, New York, Kansas, Texas, and the District of Columbia, and it has been reported in Europe, South America, and Asia.

**Incidence of Infection.**—There are no exact data available as to the

incidence of infection with *Trichomonas buccalis*, but I believe that it is a comparatively common inhabitant of the human mouth. I have encountered it several times in making examinations for *Endamæba gingivalis* and also during routine examinations of the mouth during class work in protozoology.

**Method of Transmission.**—The method of transmission of this flagellate is unknown, but it is probably through the medium of contaminated dishes and drinking utensils.

**Experimental Infection of Lower Animals.**—None of the lower animals have been successfully infected with this flagellate.

**Relation to Disease.**—There is no evidence that *Trichomonas buccalis* is a pathogenic flagellate. Under certain conditions it may possibly aggravate an existing inflammation of the mucous membrane of the gums or mouth, but it apparently has no connection with any of the pathological conditions with which it may be associated, so far as etiology is concerned.

**Prophylaxis.**—Prophylaxis consists in proper care of the teeth and mouth.

**Doubtful Species.**—In addition to the trichomonads already described, the trichomonad of the lung described by Schmidt, in 1895, should be mentioned. He called this trichomonad, *Trichomonas pulmonalis*, but his description was so inadequate as regards specific features that it is impossible to accurately classify the organism. While, in all probability, *Trichomonas pulmonalis* is identical with *Trichomonas buccalis*, it must be regarded at present as impossible of identification.

**Free-living Flagellates in the Mouth.**—When conditions are favorable there is no reason why free-living flagellates might not occur as transient inhabitants of the human mouth. One such flagellate has recently been described by Knowles and Das Gupta (1924) as occurring in the saliva of a patient observed in Calcutta. These authors did not name the flagellate, but stated that it belonged to the genus *Bodo*. I suggest for this flagellate the name, *Bodo salivarius*.

BODO SALIVARIUS (Knowles and Das Gupta, 1922), sp. nov.

This parasite was found by Knowles and Das Gupta (1922) in the sputum of a patient suffering from spleno-medullary leukæmia, and which they later determined to be present in the saliva. It occurred on August 18 and 19, and again on October 27, but later examinations were negative. The following data are taken from their description of the organism.

The length varies from 8 to 12 microns and the breadth from 5 to 9 microns. The organism is oval or pear-shaped and has two flagella, a short thick anterior flagellum and a long thin posterior flagellum. There is a large oval or spherical nucleus and a small, deeply stained dot

situated at the point of origin of the flagella, which appears to be a kinetonucleus. No cytostome or contractile vacuole could be distinguished in the living specimens, but in some of the stained specimens appearances suggestive of a contractile vacuole were noted. The organism was actively motile, moving about in the jerky manner characteristic of flagellates belonging to the genus *Bodo*. No cysts were observed.

Cultivation was successful in Row's hæmoglobin medium, at 22° and 37° C. The cultural forms were more rounded and vacuolated than the forms found in the saliva.

Knowles and Das Gupta state that the organism "appeared as a merely transient infection in the mouth, presumably during a phase when environmental conditions were suitable; it is in no wise pathogenic, but a harmless commensal."

They believe

that the nearest human analogue is *Bodo edax*, which occurs as a coprozoic flagellate in the fæces of man.

The Diagnosis of the Intestinal Flagellates and the Flagellates of

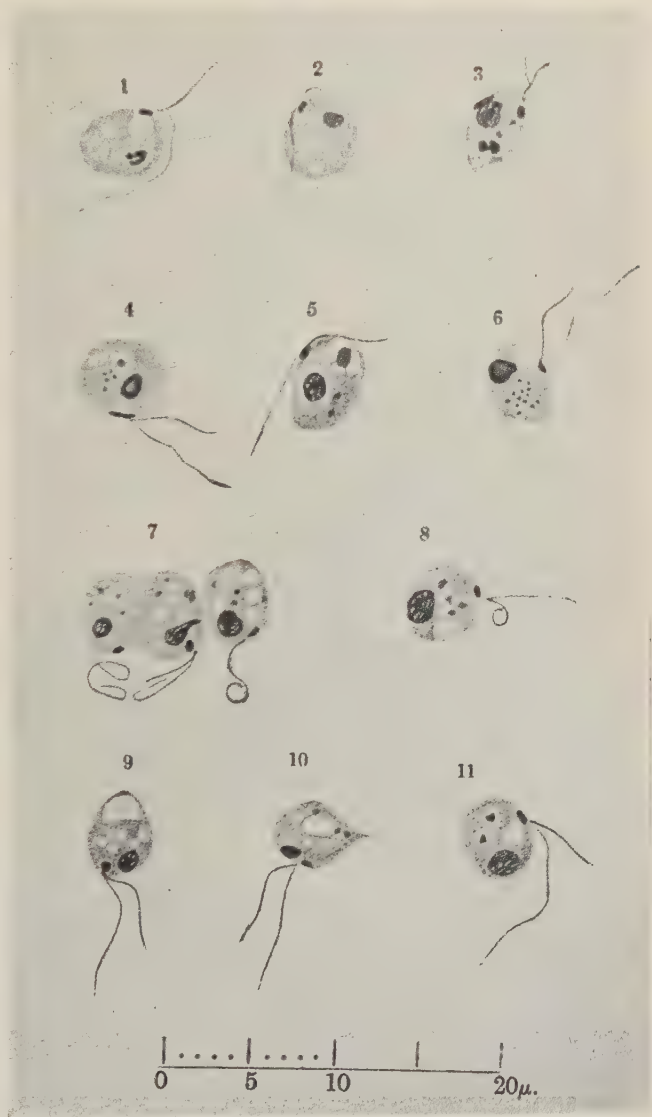


FIG. 44.—*Bodo salivarius*. Forms observed in cultures. (After Knowles and Das Gupta.)

**the Mouth and Vagina.**—The diagnosis of the intestinal flagellates rests upon their demonstration in the fæces. For this purpose either fresh or stained preparations may be used, the best results, of course, being obtained with well-stained preparations. Wet-fixation and staining with iron hæmatoxylin or some other hæmatoxylin stain gives the best results, but very beautiful specimens may sometimes be obtained by staining with the Wright or Giemsa stain. For directions regarding the use of these stains in the diagnosis of the flagellates the reader is referred to the Appendix.

The examination of the freshly passed stool is very useful in the diagnosis of the intestinal flagellates and should never be neglected. A small amount of fæces is mixed with normal salt solution or distilled water and placed upon a microscopic slide and gently flattened out with a cover-slip. Most of the intestinal flagellates can be easily picked up with the low-power (16 mm.) objective, but little can be learned of their structure unless the high-power dry objective (4 mm.) be used, and in most instances the oil-immersion objective should also be used in the diagnosis of these parasites. Careful cutting down of the light is necessary in order to bring out the structure, and organelles like flagella and undulating membranes cannot be seen until the parasite has almost ceased motion. It is perfectly possible to make a differential diagnosis of most of the intestinal flagellates in unstained preparations, and this is the method of choice for the clinician. Careful attention to morphology and comparisons of what is found with the descriptions of the various parasites described should enable one to diagnose these organisms without any great difficulty in unstained preparations, but for the differentiation of some of the species of the smaller flagellates stained preparations are usually required.

The cysts of the various intestinal flagellates are easily distinguished after a little practice, and it should be remembered that they occur most frequently in the formed stools, while the motile vegetative stages of these organisms are found only in liquid or semi-fluid stools. Therefore, it is necessary, in many instances, if one desires to study the latter forms, to administer a saline cathartic and examine the first liquid or semi-fluid stool that is passed after its administration.

The importance of examining freshly passed stools cannot be over-emphasized, for stools easily become contaminated with coprozoic flagellates, especially in subtropical and tropical regions, and many mistakes have been made in diagnosis due to examining stale stools which contained these flagellates.

The flagellates of the mouth and vagina can be demonstrated in either fresh or stained preparations. *Trichomonas buccalis* is present in the material around carious teeth or in the pus pockets in pyorrhea alveolaris,



and a little of this material should be placed upon a slide and examined under a cover-glass. *Trichomonas vaginalis* is found in the acid secretions from the vagina, and all that is necessary in order to demonstrate its presence is to examine the vaginal secretions in the same manner as already described for examination of the fæces. More detailed directions for the examination of the fæces will be found in Chapter V, treating of the diagnosis of the intestinal amœbæ.

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## CHAPTER VIII

### THE BLOOD AND TISSUE FLAGELLATES OF MAN. THE TRYPANOSOMES. GENERAL DESCRIPTION. *TRYPANOSOMA GAMBIENSE*. *TRYPANOSOMA RHODESIENSE*. DIAGNOSIS OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE*.

Two very important groups of the FLAGELLATA inhabit the blood and tissues of man, the *Trypanosomes* and the *Leishmania*.

The *Trypanosomes* are protozoan organisms belonging to the FLAGELLATA, family TRYPANOSOMIDÆ, and are the cause of serious diseases both in man and domestic animals. Species are found very widely distributed in nature, in both vertebrates and invertebrates, and the majority of the species which have been described are apparently non-pathogenic parasites, but the species occurring in man are all pathogenic.

**History.**—Velentin (1841), of Berne, discovered and described the first trypanosome of which we have knowledge, a parasite of the brown trout, and his discovery was followed by the discovery, by Gluge, of a trypanosome in the blood of the frog, and by Gros (1845) of a trypanosome in the blood of the field-mouse and mole. In 1850, Wedl found similar parasites in the blood of birds, and in 1871, Ray Lankester found trypanosomes in the blood of batrachians. Interest in these organisms was renewed in 1871, when Lewis found that a trypanosome was a common parasite of the rat in India, and his observations were soon confirmed by workers in other parts of the world. All of the trypanosomes discovered up to and including Lewis's trypanosome were non-pathogenic, but in 1880, Evans discovered a trypanosome in the blood of a horse suffering from a disease called "surra," and which he proved to be the cause of the disease. The discovery of Evans was followed by that of Rouget (1894) of the trypanosome causing dourine; of Bruce (1895), of the trypanosome causing nagana; and of Elmassian (1901), of the trypanosome causing mal de caderas.

Until 1901, it was supposed that the trypanosomes were parasites of the lower animals only, but in that year Forde discovered, in the blood of a European in the hospital at Bathurst, Gambia, minute, actively motile, worm-like bodies, the nature of which he was unable to determine, but Dutton, who examined the blood of the same patient in December, 1901, recognized the bodies as trypanosomes and gave the name *Trypanosoma gambiense* to the organism. In 1902, Dutton and Todd observed the same parasite in several other patients in Gambia presenting irregular fever, and in 1903, the presence of this parasite in man was confirmed by Manson, Broden, Brumpton, and others.

In 1903, Castellani, while engaged in examining the cerebrospinal fluid of negroes suffering from sleeping sickness, discovered that a large proportion of them showed a trypanosome in this fluid, which he considered a different species from *Trypanosoma gambiense*, and which he called *Trypanosoma ugandense*, a name afterward changed to *Trypanosoma castellanii* by Kruse. The discovery of Castellani was at once confirmed by Bruce and Nabarro, who found the parasite in every one of thirty-eight cases of sleeping sickness, and in twelve of them in the peripheral blood. Bruce and Nabarro considered the trypanosome as the cause of the disease and their observations were confirmed by Greig, Brumpt, Dutton, Todd, Broden, and Christy.

For some time the trypanosome found in the cerebrospinal fluid in sleeping sickness and that found in the blood in patients suffering from irregular fever were thought to be different species, but the observations of Bruce and Nabarro, Dutton and Todd, and others, proved beyond doubt that the trypanosome found in sleeping sickness, and discovered by Castellani, was identical with that found in fever cases, and discovered by Forde and Dutton. The later observations of Thomas and Linton, Laveran, Thomas and Breinl, and Gray and Tulloch upon the pathological lesions produced in animals by these organisms confirm the observations based upon morphological and experimental evidence, and it is now generally accepted that *Trypanosoma gambiense* and *Trypanosoma ugandense* are identical and that the proper name of the parasite is *Trypanosoma gambiense*. Castellani alone, of modern students of the trypanosomes, adheres to the belief that the trypanosome found in the cerebrospinal fluid in sleeping sickness is a species distinct from the organism found in the blood in fever cases.

In 1903, both Sambon and Brumpt advocated the theory that the trypanosome causing sleeping sickness was transmitted from man to man by the tse-tse fly, the species concerned being *Glossina palpalis*, and Bruce and Nabarro proved experimentally that the infection could be transmitted by this fly. They believed that the transmission was purely mechanical, but Kleine (1909) demonstrated that the trypanosome underwent a period of development in the fly, and other observers have described sexual forms in infected flies.

In 1909, Chagas described a new species of trypanosome in man in South America and proved that it was transmitted from man to man by a biting insect, *Lamprophya megistus*, and in 1910, Stephens and Fantham described still another species occurring in man in Rhodesia, calling it *Trypanosoma rhodesiense*, and Kinghorn and Yorke (1912) proved that this species is transmitted from man to man by a biting fly, *Glossina morsitans*.

**Morphology of Trypanosomes.**—Trypanosomes are generally spindle-

shaped, consisting of a mass of cytoplasm which contains a large nucleus, the macronucleus or trophonucleus, and a smaller body, the blepharoplast or kintonucleus. The latter is united, by a delicate filament, with a minute granule, the basal granule, which, by some authors, is called the blepharoplast. The delicate filament uniting the blepharoplast, or kintonucleus, with the basal granule, is called the rhizoplast. There is an undulating membrane, the outer edge of which is formed by a flagellum arising from the blepharoplast, and which terminates as a free flagellum or ends with the undulating membrane in the body of the trypanosome without becoming free, at the opposite end of the body from its point of origin.

Among protozoologists there is still a difference of opinion as to which end of a trypanosome the flagellum arises from, the anterior or posterior. Laveran and Mesnil consider the end of the organism from which the flagellum originates as the posterior extremity and the end from which it projects as a free flagellum as the anterior, but Woodcock, Sambon, Castellani, and others regard the extremity from which the flagellum projects as the posterior extremity. As a matter of fact, trypanosomes may move with either extremity in advance, and most authorities now accept the flagellated end as the anterior end of the parasite. However, in order to avoid confusion I shall use the terms *flagellate* and *non-flagellate* end in the descriptions of the trypanosomes that follow, the flagellate extremity being the one from which the flagellum projects as a free flagellum.

In the living condition trypanosomes are colorless, very active organisms, and it is impossible to make out their structure unless specimens are fixed and stained appropriately. Under the dark field the flagellum and undulating membrane can be easily distinguished and the character of the movements of these structures and of the organism as a whole can be studied to advantage.

The trypanosomes present for description a body, an undulating membrane, and a flagellum. In stained preparations all of the details of structure can be well studied, especially if the preparations be wet-fixed and stained with iron hæmatoxylin, but beautiful preparations, in which all of the essential details of structure can be distinguished, are secured by staining with the Wright or Leishman stain after dry fixation.

The *body* of a trypanosome is more or less spindle-shaped and consists of a mass of cytoplasm, the endoplasm, surrounded by a delicate periplast or ectoplasm. Some species of trypanosomes are almost pear-shaped or rounded in contour, and the shape of the organisms may vary with the species of animal infected.

The flagellate extremity of the trypanosome is generally longer and more narrow than the non-flagellate extremity, and a careful exami-



nation will demonstrate that it is usually continued for a little distance along the flagellum, which thus does not become free directly from the body, but gradually, as a continuation of the undulating membrane and the cytoplasm of the body.

The non-flagellate extremity varies extremely in shape in different species, in individuals of the same species, and in the same species in the blood of different animals. Thus it may be elongated and pointed, short and blunt, or short and pointed.

The cytoplasm of trypanosomes stains blue when the Wright or Leishman stain is used and is composed apparently of fine granules arranged in irregular masses. This arrangement of the granules causes an irregular staining of the cytoplasm, some areas appearing deep blue, while others are almost unstained. Metachromatic granules are present in some species, staining a deep violet or almost black, and irregularly distributed in the cytoplasm. These granules have been used in the determination of species, in some instances, but are really not of great service in this direction. One or several vacuoles may be present in the cytoplasm, but there is never a contractile vacuole. Degenerating organisms may be filled with vacuoles of various size.

The cytoplasm contains a large nucleus, the trophonucleus, which stains a rose-red or violet with the Wright stain, and which is generally situated at or near the centre of the body. However, it may be situated at either end in different species, and the situation of the nucleus is of some service in the differentiation of species. With the Wright stain the nucleus appears to be composed of chromatin granules arranged in a compact manner and surrounded by a dimly staining nuclear membrane. In wet-fixed specimens stained with iron hæmatoxylin the nucleus shows a well-defined nuclear membrane and a central karyosome.

The cytoplasm of the body also contains a small oval or rod-shaped body, the blepharoplast, often incorrectly called the kinetonucleus. In most species of trypanosomes this body is situated at or near the non-flagellate end of the body, but it may be situated near the trophonucleus or even near the flagellate end. It generally consists of a very tiny collection of fine granules, staining so intensely that the separate granules of which it is composed cannot be differentiated. It stains deep violet or almost black with the Wright stain and is generally surrounded by an unstained halo which may represent a vesicle. It measures from 0.5 to 1 micron in diameter.

Extending from the blepharoplast a dimly stained fibril may be seen in some trypanosomes, which ends in a minute dot or granule, called the basal granule, and which is called by some authors the blepharoplast. The fibril is called the rhizoplast and is seldom clearly differentiated, the basal granule appearing as a minute dot near the blepharoplast. In

many trypanosomes the blepharoplast and basal granule cannot be differentiated, in which case the flagellum appears to arise from the blepharoplast, but when the basal granule is visible it will be seen that the flagellum really arises from this granule.

The undulating membrane is a beautifully curved and delicate membrane extending as a crest or ridge, formed of ectoplasm, for a variable distance along the body of the trypanosome. It varies in length with the situation of the blepharoplast or basal granule, and as these structures are generally near the non-flagellate extremity, the undulating membrane extends along the greater portion of the body. It is formed of the ectoplasm, but the free border is thickened by the flagellum, which forms the outer border of the undulating membrane. The membrane always arises opposite the blepharoplast on the lateral surface of the body and extends toward the flagellar extremity, where it forms, in conjunction with the flagellum, the flagellate extremity of the body. The number of curves in the undulating membrane varies somewhat with the species of trypanosome and in some species the undulating membrane is almost straight. The presence of myonemes, or striations, in this membrane has been noted in certain species, but in most mammalian trypanosomes the membrane does not present any definite structure. With the Wright stain the undulating membrane stains a bluish color, the border staining red, as it is formed by the flagellum.

The flagellum arises from the basal granule, when this is visible, or from the blepharoplast when it is not, and passing through the endoplasm to the ectoplasm, raises the latter into the beginning of the undulating membrane. From here the flagellum runs toward the flagellate extremity, forming the free, or outer, edge of the undulating membrane and projecting, in most instances, as a free flagellum from the flagellate extremity of the body. With the Wright stain the flagellum stains a bright pink, red, or violet color and is easily distinguished. For convenience of description the flagellum is divided into three parts, the first part being that portion extending from the point of origin to the commencement of the undulating membrane, and called the root; the second part being the portion running along the edge of the undulating membrane; and the third part, that portion forming the free flagellum. The thickness of the flagellum varies, it being thickest along the border of the undulating membrane and thinnest at its origin and its free extremity. The length of the free extremity also varies in different species and in different individuals of the same species.

The movements of the trypanosome are rendered possible by the undulating membrane and the flagellum.

**Life-history.**—The life-history of trypanosomes is characterized by an alternation of hosts, one generation being generally completed in the

blood of a vertebrate host and the other in the alimentary tract and appendages of a blood-sucking invertebrate host.

The life-cycle in the vertebrate host has been incompletely worked out by several observers. After infection by the bite of the invertebrate host, there follows a period in which the trypanosomes cannot be found in the peripheral blood, this period being known as the incubation period. Fantham has found small round forms in the blood during this period which are supposed to be multiplication parent forms of the organisms which are finally found in the blood. After a period of several days trypanosomes begin to appear in the peripheral blood of the vertebrate host and these forms vary much in size and morphology. Several observers have described at this time what they regarded as sexual forms in the peripheral blood, but at the present time sexual forms are not recognized, and the pleomorphism noted is regarded as merely different stages in the development and growth of the organisms in the blood.

The multiplication of trypanosomes in the blood of the vertebrate host occurs by binary longitudinal division, but schizogony, or multiple division, may occur. Division is by amitotic division of the blepharoplast, basal granule, and trophonucleus, and the formation of a new flagellum in the daughter organism and the eventual division of the cytoplasm. According to Castellani and Chalmers, the details of the process of binary fission in trypanosomes are as follows:

"1. The kinetonucleus swells up and forms a vesicle through which the chromatin is evenly distributed.

"2. The chromatin forms a band across the middle of the vesicle.

"3. The band elongates and divides into two portions.

"4. The two portions move apart, all trace of the vesicle disappears, and the two kinetonuclei are formed.

"5. The blepharoplast divides at the same time as the kinetonucleus.

"6. Either the old flagellum divides or a new flagellum develops from one of the new blepharoplasts.

"7. The central karyosome of the trophonucleus either divides and the two portions move to opposite poles of the nucleus, but are connected by a fine line, or the chromatin forms an equatorial plate which divides transversely and each half goes to an opposite pole, or the chromatin gathers around opposite poles.

"8. The connecting line disappears and two new trophonuclei are formed."

I have been able to trace every step of this process, as described by these authors, in preparations of *Trypanosoma lewisi*, and have also observed most of these divisional forms in *Trypanosoma gambiense*.

In multiple division, or schizogony, the so-called rosettes are formed, in which several organisms lie in a rosette formation, the flagella being directed toward the centre of the rosette. In this type of multiplication the trophonucleus undergoes reduction, the blepharoplast gives off a body which fuses with the trophonucleus, and both divide to form fusion masses containing from two to eight or more parasites which eventually develop into larger forms and complete the development.

In the *invertebrate* host the life-history has not been entirely ascertained and there are many different interpretations of the forms that have been found in the alimentary tract of the various insect hosts of different trypanosomes. Some authorities described sexual forms in

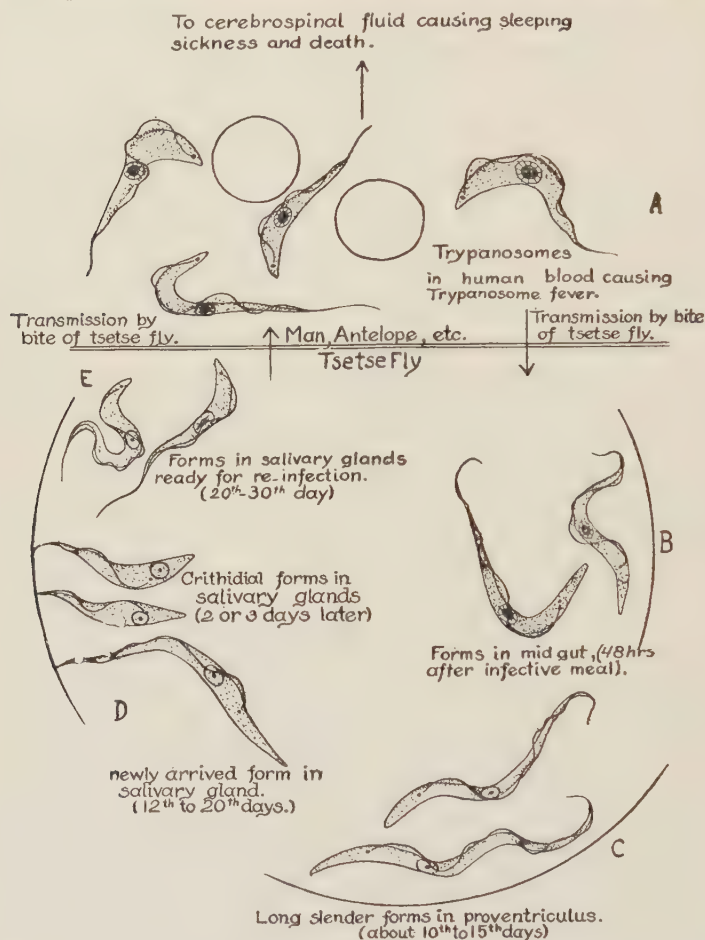


FIG. 45.—Life-cycle of *Trypanosoma gambiense*.  $\times 1,500$ . (Constructed by Chandler from figures by Miss Robertson.)

the invertebrate host, while others deny that such forms have been demonstrated. Actual observation has shown that there are three distinct forms of trypanosomes occurring in the invertebrate cycle which are accepted as being male, female, and indifferent forms by those believing in the sexual cycle of development, the male forms being long, slender organisms, the females broad and rounded, and the indifferent forms being those having a very granular cytoplasm and poorly defined nucleus and varying considerably in size and shape. However, intermediate forms be-



tween the three forms described are noted constantly and many authorities believe that the so-called sexual forms are merely stages in the development of the trypanosome in the invertebrate and that there is no sexual differentiation.

**Cultivation.**—A trypanosome was first successfully cultivated by Novy and MacNeal, in 1903, when they were successful in cultivating *Trypanosoma lewisi* in the water of condensation of tubes of the N.N.N. medium, obtaining cultures at 37° C. and at room temperature. Following their work, numerous other trypanosomes have been cultivated, the most important being *Trypanosoma brucei*, by Novy and MacNeal and Laveran and Mesnil; *Trypanosoma equinum*, by R. Thomas and Breinl; *Trypanosoma equiperdum*, by Thomas and Breinl; *Trypanosoma gambiense*, by Thomas, Breinl, Gray, and Tulloch; *Trypanosoma evansi*, by Novy, MacNeal, and Hare; and *Schizotrypanum cruzi*, by Chagas.

**Method of Transmission of Trypanosomes.**—Trypanosomes are transmitted from the invertebrate host to the vertebrate host either mechanically or through the inoculation, by biting, of forms that have developed in the invertebrate host. In the first case, direct transmission, the invertebrate host, which has become infective through the sucking of the blood of an infected vertebrate host, may inoculate the trypanosome directly into the wound made by biting, no development of the parasite having occurred in the invertebrate host. Direct infection in this manner may occur for a period of about twenty-four hours after the invertebrate host has bitten an infected individual. After this period the invertebrate host is not infective for a period of several days, which varies in different hosts and with different trypanosomes, but after this period has passed the invertebrate host is again infective and remains so indefinitely. In this instance, it is evident that some development must have occurred in the invertebrate host, and we are justified in stating that a part of the life-cycle of trypanosomes has been passed in this host. The trypanosomes taken into the stomach of the invertebrate develop in the fore, mid, and hind guts, and eventually reach the salivary glands, at which time the insect becomes infective.

Some authorities have suggested that transmission of trypanosomes may occur through swallowing encysted organisms, but there is absolutely no evidence that such a method of transmission ever occurs in man or other animals.

**Pathogenicity of Trypanosomes.**—It is probably true that the majority of the species of trypanosomes that are known are non-pathogenic, and many vertebrates are parasitized by organisms belonging to this genus that never show any symptoms of infection. In fact, there is no better instance in nature of the mutual balance maintained between a parasite and its host than is furnished by this class of PROTOZOA. For

instance, the well-known rat trypanosome, *Trypanosoma lewisi*, although it may be present in countless numbers in the blood of its vertebrate host, the rat, does not produce any symptoms of infection, and the host and parasite live together indefinitely without such symptoms ever appearing. Likewise, as shown by Crawley (1909), American cattle are parasitized by a trypanosome, *Trypanosoma americanum*, which occurs in the peripheral blood in such small numbers that it is necessary to culture the blood to demonstrate the organism, and which does not produce any symptoms of infection. Instances of this kind could be multiplied almost indefinitely, and in many hosts, both invertebrate and vertebrate, no symptoms are ever detected that would indicate the presence of the parasite. The most striking example of the existence of a known pathogenic trypanosome in a vertebrate host without the production of symptoms is in the case of the wild antelope in Africa and *Trypanosoma brucei*.

Although many trypanosomes are non-pathogenic, there are many species that are known to produce severe and fatal disease in their vertebrate hosts. From the standpoint of medicine, the most important of these are *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, and *Schizotrypanum cruzi*, all of which are the cause of important infectious diseases in man, their vertebrate host. In veterinary medicine a number of pathogenic trypanosomes have been discovered which produce important diseases in animals, and a list of the most important will be found on page 233.

It is a singular fact that pathogenic trypanosomes are almost without exception fatal to their vertebrate host, and some of the most terribly fatal of known infections are caused by this group of parasites. Sleeping sickness in man; surra, dourine, mal de caderas, and nagana in horses; and nagana, souma, and Sudan cattle-sickness in cattle are all deadly diseases due to species of trypanosomes.

The pathology of infection with trypanosomes causing disease in man will be discussed in the description of the parasites.

**Classification of Trypanosomes.**—The classification of trypanosomes, especially the identification and placing of the different species that have been described, is exceedingly difficult, owing to the fact that many species that are evidently distinct are alike morphologically, and also to the fact that a trypanosome that is pathogenic for one animal may be harmless for another, so that animal experimentation does not always assist us in the differentiation of species that are morphologically similar. Despite these difficulties, however, a large number of species have been differentiated that are accepted by all workers, and the species that are of importance in human pathology are included among those that have been definitely classified.

There are two genera of trypanosomes that are accepted by most proto-

zoologists, *Trypanosoma* and *Schizotrypanum*, and, so far as human pathology is concerned, these are the only genera that need to be considered. More elaborate generic classifications have been proposed, the most recent of which is that of Castellani and Chalmers (1920), who divide the trypanosomes into those infecting invertebrata, cold-blooded vertebrates, and warm-blooded vertebrates, and further into those living in a definitive invertebrate host, those living in a definitive invertebrate host and in a cold-blood intermediate vertebrate host, and those living in a definitive invertebrate host and a warm-blooded intermediate vertebrate host. Several genera are included by these authors in the three classes, and in the third group, which includes all of the trypanosomes of man and other warm-blooded animals, no less than six genera are named, *i.e.*, *Lewissonella*, *Endotrypanum*, *Trypanosoma*, *Schizotrypanum*, *Castellanella*, and *Duttonella*.

While there is no doubt that even the morphological variations met with between different trypanosomes are enough to justify the founding of several distinct genera, it is very doubtful if the differences between the pathogenic trypanosomes of warm-blooded animals are sufficient upon which to base the formation of the genera suggested by Castellani and Chalmers, and in my opinion, it is much better to recognize only two genera at present in human and veterinary medicine, *Trypanosoma* and *Schizotrypanum*, and this opinion is based upon that of the vast majority of students of the trypanosomes.

**Trypanosomes of the Lower Animals.**—As this class of parasites cause so many important infections in animals of economic interest to man, the following list of the pathogenic trypanosomes occurring in domestic animals, arranged alphabetically, may be of service:

*Trypanosoma brucei*, Plimmer and Bradford, 1899. The cause of "nagana" in horses, donkeys, mules, cattle, dogs, and cats. The disease is fatal to these animals. Probably identical species are *T. pecaudi*, *T. togolense*, *T. ugandæ*, *T. equi*, and possibly *T. rhodesiense*. Geographical distribution, Africa.

*Trypanosoma congolense*, Broden, 1904. Causes a fatal disease in cattle, horses, donkeys, and sheep, if not native to the regions in which it occurs. Probably identical species are *T. nanum*, *T. dimorphon*, *T. confusum*, *T. montgomeryi*, and *T. pecorum*.

*Trypanosoma equinum*, Voges, 1901. The cause of "mal de caderas," a fatal infection of the horse. Geographical distribution, South America.

*Trypanosoma equiperdum*, Dofflein, 1901. The cause of "dourine" in horses. Geographical distribution covers Europe, North Africa, India, and North America.

*Trypanosoma evansi*, Steel, 1885. The cause of "surra" in domesticated animals, especially the horse, mule, donkey, and camels. Cattle are also infected and the disease is a fatal one. Probably identical species are *T. annamense*, *T. berberum*, *T. elephantis*, *T. marocanum*, *T. soudanense*, *T. hippicum*, and *T. venezuelense*. (The two last-named species, *T. hippicum*, Darling, 1910, and *T. venezuelense*, Mesnil, 1910, are believed by many to be distinct species, and late work upon both appears to favor this view.) Geographical distribution practically world-wide.

*Trypanosoma guyanense*, Leger and Vienne, 1919. The cause of a fatal disease in cattle. Geographical distribution, French Guiana and Venezuela.

*Trypanosoma vivax*, Ziemann, 1905, causing fatal infection in cattle, horses, donkeys, sheep, and goats, if not native to the region in which it occurs. Probably identical species are *T. capræ*, *T. casalboui*, and *T. uniforme*. Geographical distribution, Africa.

It will be noted that many of the species named above have been rediscovered and renamed by different observers, and the literature is filled with descriptions of trypanosomes that were believed to be distinct species, but which were subsequently proven to be identical with one of the species mentioned in the list.

**The Trypanosomes of Man.**—The parasitic trypanosomes of man belong to two genera, *Trypanosoma* and *Schizotrypanum*.

### Genus I. TRYPANOSOMA, Gruby, 1843.

Synonyms: *Amaba*, Mayer, 1843. *Paramœcium*, Mayer, 1843. *Globularia*, Wedl, 1850. *Undilina*, Lankester, 1871. *Herpeiomonas*, Kent, 1878. *Hæmatomonas*, Mitrophan, 1883. *Trypanosomonas*, Danilewsky, 1885. *Trypanozoon*, Lühe, 1906. *Duttonella*, Chalmers, 1918. *Lewisonella*, Chalmers, 1918. *Castellanella*, Chalmers, 1918.

While others may have seen trypanosomes in the blood of amphibia, the first to describe a trypanosome was Valentin, in 1841, who discovered and described a species in the brown trout. The genus *Trypanosoma* was founded by Gruby, in 1843, and the type species of the genus is *Trypanosoma rotatorium*, a parasite of frogs.

In the genus *Trypanosoma* there are two species which are very important pathogenic parasites of man, *Trypanosoma gambiense* and *Trypanosoma rhodesiense*. These are the only parasites belonging to this genus that are parasitic in man, for, unlike many of the lower animals, man is not parasitized by non-pathogenic trypanosomes.

### Species I. TRYPANOSOMA GAMBIENSE, Dutton, 1902.

Synonyms: *Trypanosoma ugandense*, Castellani, 1903. *Trypanosoma hominis*, Manson, 1903. *Trypanosoma nepveui*, Sambon, 1903. *Trypanosoma castellanii*, Kruse, 1903. *Trypanosoma nigeriense*, Macfie, 1913. *Castellanella gambiensis*, Dutton, 1912, *emend.* Chalmers, 1918. *Castellanella castellanii*, Kruse, 1903, *emend.* Chalmers, 1918.

Some authorities believe that Nepveu (1898) was the first to discover this species in the blood of cases of malaria occurring in Africa, but the first accurate description was published by Dutton, in 1902.

**History and Nomenclature.**—*Trypanosoma gambiense* was first seen by Forde, in 1901, in the blood of a European patient in hospital at Bathurst, Gambia, and Dutton, in 1902, after studying the parasite in the blood of the same patient, proposed for it the name *Trypanosoma gambiense*, and it was soon recognized as the cause of an irregular fever occurring in this region. In 1903, Castellani found a trypanosome in the cerebrospinal fluid of patients suffering from sleeping sickness, and proposed for this parasite the name *Trypanosoma ugandense*, a name afterward changed to *Trypanosoma castellanii*, by Kruse, in 1903. For some time it was accepted that the trypanosome found in the cerebro-



spinal fluid in sleeping sickness was a different species from that found in the blood in cases suffering from irregular fever, but it gradually became evident that the two organisms were identical and that the patients having irregular fever were really in the first stages of sleeping sickness. That the two supposed parasites are identical has been proven by so many investigators that it is not necessary at this time to discuss the subject further. It may be stated, however, that Castellani still adheres to his original belief in the specific status of the trypanosome he found in the cerebrospinal fluid, but is practically alone in this belief.

As *Trypanosoma gambiense* and *Trypanosoma ugandense* or *Trypanosoma castellanii* are identical, the specific name "*gambiense*" becomes the proper specific name, by law of priority, and "*ugandense*" and "*castellanii*" become synonyms of "*gambiense*," and the proper name of the trypanosome becomes *Trypanosoma gambiense*.

In Chalmers' new classification of the trypanosomes, noted by Castellani and Chalmers (1920), the distinction between the trypanosome found in the cerebrospinal fluid and in the blood is still maintained, the former being called *Castellanella castellanii* and the latter *Castellanella gambiensis*. The genus *Castellanella* is not accepted by most protozoologists and it has already been stated that these supposed species are identical, so that *Castellanella gambiensis* and *Castellanella castellanii* also become synonyms of *Trypanosoma gambiense*.

Macfie, in 1913, described a trypanosome occurring in young people in Southern Nigeria, and causing a milder form of disease, which he considered a distinct species and which he called *Trypanosoma nigeriense*. Further research has shown that this organism is almost certainly identical with *Trypanosoma gambiense*, being a less virulent strain of the latter, and the name *Trypanosoma nigeriense*, therefore, becomes a synonym of *Trypanosoma gambiense*.

**Morphology.**—*Trypanosoma gambiense* is a typical trypanosome and may be distinguished in the fresh blood with a high dry objective, appearing as a spindle-shaped, colorless body, moving actively about among the corpuscles or endeavoring to force its way among them. Its morphology cannot be studied well in unstained preparations, but blood smears or preparations of the sediment from cerebrospinal fluid stained with the Wright or Leishman stain will show all of the essential ele-

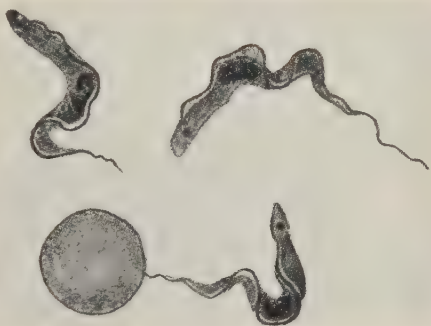


FIG. 46.—*Trypanosoma gambiense*.  $\times 1,700$ .  
(After Dutton.)

ments of the morphology of this parasite. For fine nuclear details wet-fixation and staining with iron hæmatoxylin gives the best results. The staining reactions are those already noted in the general description of the trypanosomes.

The length of the parasite, in stained preparations, varies considerably, as this organism is polymorphic, and long, slender forms as well as short and medium forms occur in the same preparation. Laveran and Mesnil (1907) give the length as 17 to 28 microns and the breadth

as 1.4 to 2 microns, while Castellani and Chalmers give the length as from 14 to 33 microns and the breadth as from 2 to 2.5 microns. In my experience the length of this species has varied from 16 to 30 microns and the breadth from 1.5 to 2.5 microns. The longest forms are those that are undergoing division, as a rule.

The trypanosome is roughly spindle-shaped, but short and more rounded forms occur. The non-flagellate end is generally narrow and pointed, but often broader and rounded. The flagellate end is

FIG. 47.—*Trypanosoma gambiense*.  $\times 1,400$ .  
(Photomicrograph. Army Medical School Collection.)  
Wright's stain.



attenuated and pointed, gradually blending with the termination of the undulating membrane and with the free portion of the flagellum.

The body of the parasite contains an oval trophonucleus, situated at or near the centre of the body and staining deeply with the chromatin stains, and a blepharoplast, consisting of a small deeply stained dot of chromatin, situated in the non-flagellate extremity of the body. In some very well stained preparations a distinct basal granule, situated close to the blepharoplast, can be distinguished, but in the vast majority of the specimens the basal granule and blepharoplast are blended.

The flagellum arises from the basal granule, when the latter can be distinguished, but apparently from the blepharoplast, in ordinary specimens, runs toward the flagellate end of the parasite, forming the outer edge of the undulating membrane, and terminates as a free flagellum which forms from one-quarter to nearly one-half the total length of the organism. In rare instances the flagellum appears to terminate at the flagellate end, and in the short, stumpy forms there is often no free flagellum or it is very short.

The undulating membrane originates near the blepharoplast and ter-

minates by merging into the body of the organism at the flagellate extremity. The membrane is broad and thrown into folds, the outer border being formed by the flagellum. It is composed of cytoplasm and does not show any evidence of the presence of striations or myonemes.

The body may contain vacuoles and deeply staining metachromatic granules.

The description given applies to the majority of the trypanosomes of this species, as observed in the blood or cerebrospinal fluid of man, but *Trypanosoma gambiense* is a very polymorphic species and great variations may be noted in its morphology in stained preparations, some of them probably due to the staining process and others representing stages in the growth and development of the parasite. Long, slender forms and short, blunt ones are observed in the same specimen; some that stain well, and others that stain poorly; some in which the basal granule and blepharoplast are distinct, but more in which both of these structures are represented by a single deeply stained dot, which may, or may not, lie within an unstained oval or spherical area, resembling a vacuole. Forms also occur without a free flagellum and others in which the undulating membrane is rudimentary or even absent. In some, metachromatic granules are absent, while in others such granules may be numerous. Degenerating forms are also frequently encountered which are much vacuolated and stain poorly.

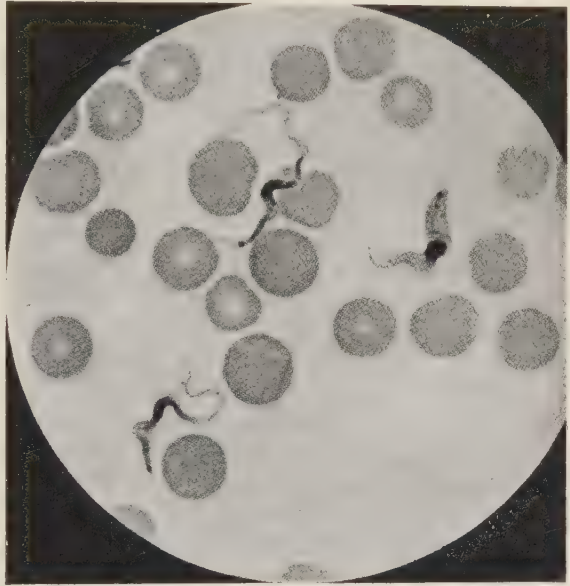


FIG. 48.—The three forms of *Trypanosoma gambiense* observed in the blood of man. Photomicrograph. (Minchin.) This photomicrograph shows the long and slender, short and broad, and intermediate types of *T. gambiense* that are observed in the blood and that were formerly believed to be sexual forms.

In most infections two distinct forms of *Trypanosoma gambiense* may be distinguished, one, long and slender, and having a free flagellum of considerable length, and the other, short and broad, and having a very short free flagellum. The long forms were held by German investigators to be males and the short forms females, but the researches of Robertson (1912), which have been confirmed by many other inves-

tigators, prove that these forms are not sexual, but merely stages in the growth of the trypanosomes.

Dividing forms are observed in the blood in which there are two blepharoplasts, two nuclei, and a division of a whole or part of the flagellum has occurred. Other forms are seen in which two perfectly formed trypanosomes are still held together by a portion of the cytoplasm, and intermediate forms are noted showing every stage of binary longitudinal division. The forms resulting from this division may be equal in size or unequal, one being much larger than the other.

**Morphology of *Trypanosoma gambiense* in *Glossina palpalis*.—**The morphology of *Trypanosoma gambiense* in its intermediate host, the tse-tse fly, *Glossina palpalis*, has been very carefully studied by Robertson (1912) and others, and will be described in the discussion of the life-history of this species. During the development of the parasites in the fly, very marked variations occur in the morphology, and in the salivary glands of an infected fly all stages, from typical crithidial forms to fully developed trypanosomes, as seen in the blood of man, may be observed.

**Latent Forms in Man.**—Fantham (1911) described "latent forms" in the spleen, liver, lungs, and bone-marrow, which are not flagellated, but which, under certain conditions, may become flagellated and develop into the typical trypanosome, again appearing in the peripheral blood. Such forms are oval in shape and contain the nucleus and blepharoplast, but no undulating membrane or flagellum. Laveran (1911) and others regard these as "involution forms," but it has been proven that these forms can flagellate and appear in the peripheral blood, and that they are infective.

**Habitat.**—*Trypanosoma gambiense* lives and multiplies in the blood of man and is found in the cerebrospinal fluid in the terminal stages of sleeping sickness. It does not undergo any development in the cells of the internal organs, but latent forms have been described as occurring in the spleen, liver, lungs, and bone-marrow. Recent investigations by Yorke (1911) and Wolbach and Binger (1912) have shown that *Trypanosoma gambiense* also occurs in the connective tissue of all the organs, the reticular tissue of the lymph-nodes and spleen, and the nervous tissue, and in these locations the parasites produce lesions that are characteristic of the infection.

**Species Occurring in Lower Animals.**—*Trypanosoma gambiense* has been found in a species of antelope, the situtunga, *Tragelaphus spekei*, on an island in Victoria Nyanza, by Duke (1912), and the tse-tse fly, *Glossina palpalis*, was infected experimentally from these animals. It would thus appear that the antelope might act as a reservoir of infection for man.

As is well known, the tse-tse fly, *Glossina palpalis*, is naturally infected and *Glossina morsitans*, as well as other species of this genus, may be



experimentally infected with *Trypanosoma gambiense* and act as transmitting agents of the infection to man.

**Cultivation.**—Novy and MacNeal (1903) were successful in cultivating *Trypanosoma brucei* upon the N.N.N. medium, and their success with this closely related trypanosome led to efforts to cultivate *Trypanosoma gambiense* which have met with only partial success. Thomas and Breinl (1905) were able to keep the trypanosome alive for as long as 68 days in a medium composed of chicken and veal infusion containing 1 to 2 per cent. salt, 2.5 to 3.5 per cent. agar, and 1 to 1.5 per cent. peptone, added in the proportion of two to one, or three to two, to rabbit's or sheep's blood, rabbit's blood being preferable. While the parasites remained alive on this medium for the period mentioned, most of them died within thirty days and multiplication did not occur.

Gray and Tulloch (1907), using the N.N.N. medium containing defibrinated dog's blood, were able to secure multiplication in their cultures, but were not able to secure subcultures. In their culture tubes, which were inoculated from the blood of a white rat infected with *Trypanosoma gambiense*, there were found on the fifteenth day numerous trypanosomes, many of which were undergoing division. The trypanosomes were longer and broader than those observed in the blood of the rat and of man, and the blepharoplast was much closer to the nucleus. After the twentieth day the trypanosomes disappeared from the cultures.

Thomson and Sinton (1912) cultivated *Trypanosoma gambiense* on a modified N.N.N. medium at a temperature of 22° to 24° C. for a period of thirty-seven days and found that the forms occurring in the cultures were similar to those found in infected tse-tse flies.

While experimental infections may be produced with cultures of *Trypanosoma gambiense*, it has been determined that such experiments are not attended with success unless the cultures are less than four days old and, in most instances, the cultures are not infective after three days.

**Life-history.**—*Trypanosoma gambiense* develops both in the blood of man and in the alimentary tract and salivary glands of tse-tse flies. The flies act as transmitting agents to man, but it cannot be said that the development in the fly is sexual in nature, as is the development of the plasmodia of malaria in *Anopheles* mosquitoes, for, while some authorities claim to have demonstrated sexual forms in the fly, the consensus of opinion today appears to be that the development in the fly is not sexual in type.

In *man* the life-history of *Trypanosoma gambiense* consists, so far as it has been ascertained, of periods of multiplication alternating with quiescent periods in which, in the opinion of some authorities, the organism assumes a latent form. It is well known that at intervals in infected animals and man the trypanosomes may occur in large num-

bers in the peripheral blood, while at other times the number is so small that the organism is demonstrated with the utmost difficulty. Frequently not a single trypanosome can be found in the peripheral blood, although the injection of such blood into susceptible animals produces infection. These facts have led to the theories of a sexual cycle in the development in man, of the formation of latent bodies, and of the filtrable nature of the parasite, at some stage of its development. There is no clear evidence of a



FIG. 49.—Latent forms of *Trypanosoma gambiense* and their development into trypanosomes. (After Fantham.) 1, 2 and 3. Latent forms of *T. gambiense* with remains of trypanosome around them. 4, 5 and 6. Various forms of latent bodies of *T. gambiense*. 7 and 8. Herpetomonad forms of latent bodies. 9, 10, 11, 12, 13 and 14. Crithidia-like forms of latent bodies. 15. Stout trypaniform parasite from heart blood of rat.

sexual development of this species in man, or that the organism is filtrable at any stage of its development in man, but there is very good evidence that latent forms are produced during the development cycle in man.

Robertson (1912), who has given by far the best description of the development of *Trypanosoma gambiense* in man and in the

tse-tse fly, has determined that multiplication in the blood of man is initiated by the division of the blepharoplast and basal granule, after which the flagellum splits into two parts and separates, one being generally much longer than the other. The trophonucleus shows two deeply

staining granules situated on the nuclear membrane at opposite poles of the nucleus, which apparently act as centrosomes, the nucleus constricting and each half containing one of these granules. The trophonucleus divides into two portions, which is followed by the division of the cytoplasm of the body of the organism into two daughter trypanosomes, the final division occurring at the non-flagellate extremity of the organism. The daughter trypanosomes may be equal or unequal in size and division is repeated until enormous numbers of trypanosomes occur in the blood. After a period of rapid division the trypanosomes disappear from the peripheral blood, but may be found in the enlarged lymphatic glands and in the spleen, liver, lungs, and bone-marrow, and the cerebrospinal fluid. The trypanosomes found in these localities may be typical of those occurring in the peripheral blood, but there also occur round forms,

the so-called "latent forms" of Salvin-Moore and Breinl and of Fantham.

Salvin-Moore and Breinl (1907) were the first to describe certain forms of *Trypanosoma gambiense* occurring in the spleen and bone-marrow during the intervals in which no parasites are seen in the peripheral blood, consisting of round or oval bodies without a flagellum, but containing a large and small nucleus, corresponding to the tropho-nucleus and blepharoplast of the typical trypanosome, and which, under certain conditions, developed into the typical organism as observed in the blood. Their observations were confirmed and extended by Fantham (1911), who found the forms they described in the internal organs, especially in the spleen and bone-marrow, during the time that trypanosomes were absent or few in number in the peripheral blood. The observations of Fantham were made upon rats and guinea-pigs experimentally infected with *Trypanosoma gambiense*, and he was able to observe the development of these bodies from the typical flagellate form under the microscope by mixing the blood with lymph, the entire process consuming about half an hour. The non-flagellate end of the trypanosome disintegrates, the blepharoplast migrating near the trophonucleus, while the flagellum and the flagellate end of the organism also disintegrate, leaving a rounded body enclosed in a definite capsule and containing a mass of chromatin representing the trophonucleus and a tiny granule representing the blepharoplast. In most instances the latter cannot be observed within the latent body.



FIG. 50.—Formation of latent round body from *Trypanosoma gambiense* observed in rat's blood. Time about 2 hours. (After Fantham.)

Fantham states that the latent bodies measure from 2 to 4 microns in diameter and are usually oval in shape, but may be spherical, and these bodies may sometimes be observed in the peripheral blood. Round, oval, or pyramidal bodies are also observed in the peripheral blood

which have a short flagellum, and Fantham believes these to be intermediate forms between the latent forms and the typical trypanosomes.

Fantham has not only observed the formation of the latent bodies from typical trypanosomes, but has also observed the development of typical flagellate forms from the latent bodies. He added material containing the latent bodies, obtained from the spleen of an infected rat, to normal salt solution and an equal amount of rat's blood, and observed the mixture under the microscope, the slides being kept on a warm stage at  $25^{\circ}$  to  $35^{\circ}$  C. The latent bodies became larger, sent out a

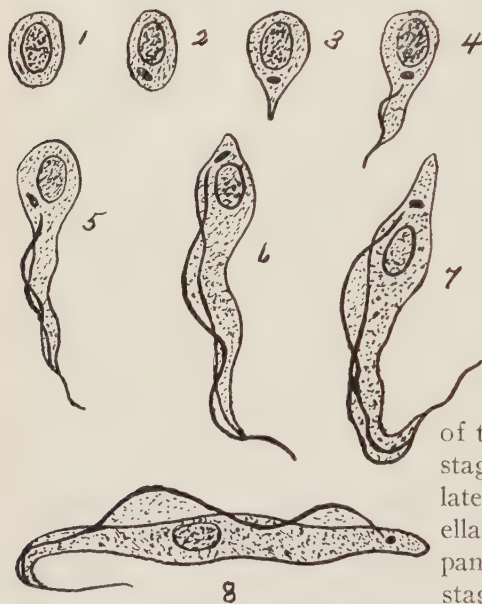


FIG. 51.—Metamorphosis of a latent round form of *Trypanosoma gambiense* into a typical trypanosome. Observed in rat's blood. Time one hour. (After Fantham.)

process which gradually lengthened, while a flagellum was formed from an area near the blepharoplast. An undulating membrane was formed along the edge of the process sent out originally and the flagellum extends and forms the outer edge of the membrane. Fantham terms this the *Crithidial* stage of development. Later the organism enlarges, the blepharoplast passes to the non-flagellate extremity of the body, and the true trypaniform stage is produced. He says: "The latent bodies, which are the post-flagellate stages of one generation of trypanosomes, become the preflagellate stages of the succeeding generation."

The latent bodies are formed at or near the period of maximum increase of trypanosomes in the peripheral blood, being especially numerous in the spleen and bone-marrow during the downward slope of the curve representing the number of parasites in the peripheral blood, while the change into the trypaniform stage occurs on the rise or upward slope of this curve. Fantham believes that these latent bodies explain the reason why infection of susceptible animals occurs after the inoculation of blood which does not contain the flagellate forms, and he has produced infection in rats by inoculating them with minute particles of splenic pulp containing the latent bodies.

Some recent writers have expressed doubt regarding the latent nature of these bodies, preferring to regard them as degenerating forms, but the evidence is quite conclusive, I believe, of their being forms vitally concerned in the life-cycle of the trypanosome in man.



It has been shown beyond doubt that *Trypanosoma gambiense* undergoes a definite life-cycle within biting flies belonging to the genus *Glossina* and that during this development forms are produced that are quite different in their morphology from those that are observed in the blood or cerebrospinal fluid of man. The development occurs most readily in *Glossina palpalis*, but also occurs in *Glossina morsitans* and other species of *Glossina*.

According to the researches of Robertson (1912) no changes occur in the trypanosomes that are taken into the alimentary tract of the fly for several days, except that the blepharoplast moves nearer to the trophonucleus and many degenerating forms occur. About the seventh to ninth days after ingestion the gut of the fly contains numerous trypanosomes of varying form, long, slender forms making their appearance about this time. Between the tenth and twentieth days the long, slender forms have increased greatly in number and work their way forward into the anterior part of the gut and the proventriculus, the latter thus becoming infected. The mid and hind gut show multitudes of multiplying forms from which the long, slender forms are produced and which thus act as a reservoir from which these forms enter the proventriculus.

The long, slender forms pass from the proventriculus into the hypopharynx and from this locality into the salivary glands, to the walls of which they attach themselves by the flagellum. After a varying period these forms become broader and resemble crithidia, the blepharoplast being situated near the trophonucleus and giving rise to a flagellum, the trophonucleus being situated toward the non-flagellate end of the body. The crithidial forms multiply rapidly in the salivary glands, and eventually trypanosomes are formed which resemble those found in the blood. A period of about five days elapses after the trypanosomes first reach the salivary glands before the fly becomes infective, and none of the forms occurring in the gut or proventriculus will produce infection in susceptible animals until the trypanosomes have reached the salivary glands.

While some authorities have described sexual forms as occurring during the development of this parasite in the fly, there is no evidence that such forms actually occur, or that the development in this insect is sexual in nature.

The development within the fly consumes from 14 to 25 days, varying considerably with the temperature and other local conditions.

**Geographical Distribution.**—The geographical distribution of *Trypanosoma gambiense* is confined to Africa and to the tropical portion of that continent. Infections occur along the west coast from Senegal on the north to Angola in the south. The regions around Lake Victoria, Lake Albert, and Lake Bangueolo are endemic centres of the infection.

French Equatorial Africa, the Belgian Congo, the Bahr-el-Ghazal region, and the islands in the Gulf of Guinea are all infected, and the infection appears to be invading the Western Mongalla and Southern Bahr-el-Ghazal region, according to Carroll (1919). The river valleys along

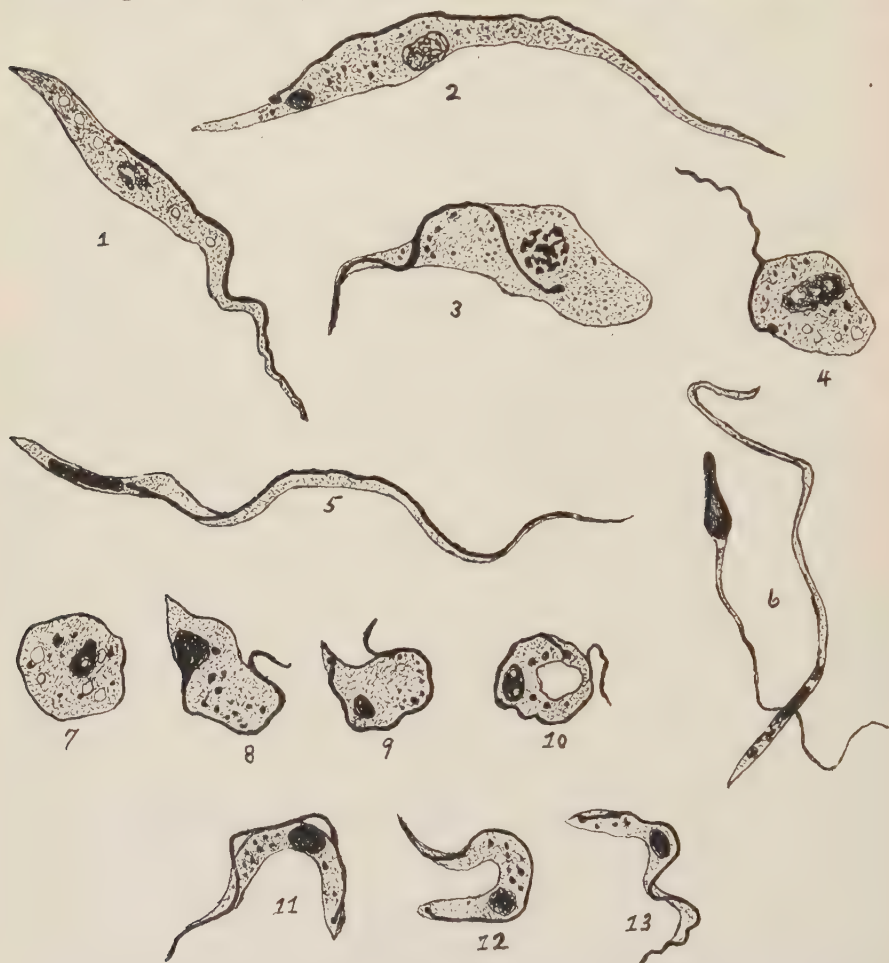


FIG. 52.—Developmental forms of *Trypanosoma gambiense* in *Glossina palpalis*. (After Kleine and Taute.) 1 and 2. Female forms in intestine of fly. 3 and 4. Broad and round forms in intestine of fly. 5 and 6. Male forms in intestine of fly. 7, 8, 9 and 10. Round stages of the trypanosome in intestine of fly. 11 and 12. Fully developed trypanosome in proboscis of fly. 13. Fully developed and typical *T. gambiense* in intestine of fly.

the west coast are badly infected, as it is along these rivers that the transmitting agent, *Glossina palpalis*, breeds and multiplies.

In Nigeria and the French, Belgian, and Portuguese Congo infections with *Trypanosoma gambiense* are common and have occurred in great epidemics which have destroyed a large proportion of the population of certain districts.

While infections with this parasite occur in all the regions which have been mentioned, it is only in certain localities in these regions that the infection is found, and these localities are strictly confined to the so-called "fly belts," or regions in which *Glossina palpalis* abounds. Intensely infected districts may be found within a comparatively short distance of districts in which only sporadic cases have been observed, and investigation has invariably proven that the freedom of the latter is due to the absence of *Glossina palpalis*.

**Incidence of Infection.**—The incidence of infection with *Trypanosoma gambiense* varies greatly in the endemic regions, some being so intensely infected that it has been necessary to remove the entire population, while in others a comparatively small number of individuals are found infected.

Blacklock and Yorke (1922) state that Greggio reported that at Kisantu, in the Belgian Congo, two-thirds of the population has perished from sleeping sickness within ten years, and that Masters, working at Leverville, in the Belgian Congo, found no less than 72 per cent. of 3,000 natives infected with this parasite. Duke (1919) states that in 1902, the population of the Buvuma Island numbered 44,311, of which 19,049 died during that year of sleeping sickness, a ratio of 4.28 per thousand, one of the highest mortality rates from an infectious disease of which we have record. Schwetz (1919), in the Belgian Congo, found in the Gobari District that 7 per cent. of infants and 6.5 per cent. of adults were infected with this parasite, and in the Kwilu District 9.5 per cent. of infants and 8.5 per cent. of adults. In the Kikwit District of the Belgian Congo, Schwetz (1921) states that 73,549 natives were examined, of which 8,922 were found infected, or\* 12.1 per cent.

In French Equatorial Africa the infection is wide-spread and the incidence high in certain localities. Jamot (1920) examined 89,743 natives and found 5,347 infected; Piot (1920), working in the Ibenga-Motaba District, examined 12,420 natives, the total population of the district, and found 1,003 infections, or 8 per cent. The infections were divided as follows: men, 3,613, or 6.2 per cent.; women, 4,789, or 5.9 per cent.; children, 4,018, or 12 per cent. Clapier (1921) states that in the Middle Congo region, in a total population of 11,417, there were 7,509 adults, of whom 259 were infected, or 3.4 per cent., and 3,908 children, of whom 734 were infected, or 18 per cent. In all except one village, one of every three children examined was found to be infected with *Trypanosoma gambiense*. He found that of 32 European soldiers who had stayed in one village for periods varying from some months to three years, seven were infected, or 21 per cent.

Muraz (1922) records the examination of 134,608 natives in French Equatorial Africa, of which number 3,071 were found infected, and

Boye (1922), in a summary of the work accomplished in French Equatorial Africa in 1921, states that 559,658 natives were examined and 28,589 were found infected, or 5.1 per cent. In some regions the infection of the natives reached as high as 50 per cent.

In the Territory of Tchad, Muraz (1920) has reported an incidence in one district of 40.5 per cent., and in three villages that he investigated the percentage of infection was 40.51, 10.94, and 8.27. These observations indicate how much the incidence of infection varies even in the same territory.

Carroll (1910), the chief medical officer at Khartoum, during 1918-1919, reports the occurrence of sleeping sickness due to *Trypanosoma gambiense* in Western Mongalla and the Southern Bahr-el-Ghazal, 341 new cases occurring in 1918, and 743 new cases in 1919. Since that time the disease has decreased in the regions mentioned due to prophylactic measures.

From the statistics that have been cited it is evident that infection with *Trypanosoma gambiense* is very common in some localities in the endemic regions and much less frequently met with in others. These differences in the incidence of infection depend upon local conditions, principally upon the number of *Glossina palpalis* present and the chances that these flies have of biting the inhabitants. In some regions the incidence of infection has been so high that depopulation has occurred through the death from sleeping sickness of practically the entire population, while in other regions it has been found necessary to remove the population to uninfected localities. In other regions the incidence of infection is so low that only sporadic cases of infection are met with and it is possible, by proper prophylactic measures, to control the disease.

**Method of Transmission.**—*Trypanosoma gambiense* is transmitted from man to man by biting flies belonging to the genus *Glossina*. The species generally concerned in the transmission of this parasite is *Glossina palpalis*, but it has been proven that it can develop in *Glossina morsitans* by Taute (1911), the Royal Society Commission (1913), and by Kleine and Fischer (1913), and it is probable that it will be found that other species of *Glossina* can transmit the parasite.

The fly may transmit the parasite *directly* from an infected to a healthy individual by infecting the wound caused by biting with material upon the proboscis, provided the insect bites within a few hours after biting the infected individual, and Bruce (1910) found that such mechanical transmission only occurs in experimental animals if the flies bite within two hours, but other authorities state that the fly remains infective for as long as twenty-four hours.

Until 1909, the year in which Kleine published his epoch-making discovery of the development cycle of *Trypanosoma gambiense* in *Glos-*



*sina palpalis*, it was generally believed that the fly always transmitted the parasite from man to man mechanically, but with Kleine's discovery the pendulum swung the other way, and for years the mechanical method of transmission was practically forgotten. However, there appears to be a tendency at present to recognize the importance of direct transmission in the etiology of sleeping sickness, through the bite of the fly, and Duke (1919), who has studied this question very carefully, and has had a large practical experience, believes that direct mechanical transmission by the fly is a most important method of transmission in certain districts, as in the Buvuna Island, already mentioned, where he believes that direct mechanical transmission was the cause of the terrible epidemics that raged there year after year.

Although direct mechanical transmission by *Glossina palpalis* may be an important method of transmission at times, and in certain regions, it is admitted that generally the fly does not transmit the infection until *Trypanosoma gambiense* has undergone a cycle of development within the insect which results in its becoming infective and remaining so indefinitely. This discovery was made by Kleine (1909), although to Bruce and Nabarro (1903) we owe our first knowledge of the possibility of the transmission of the parasite to man by the fly, having successfully produced infection in monkeys by the bites of *Glossina palpalis* that had fed upon negroes suffering from sleeping sickness.

Kleine (1909) proved that flies that have bitten a patient suffering from sleeping sickness remain negative, so far as infectivity is concerned, for a period of twenty days or more, and then become positive, thus proving that a cycle of development must occur before the trypanosomes are able to infect animals through the bite of the fly. His first work along this line was accomplished with *Trypanosoma brucei*, the cause of "nagana" in horses and cattle. He infected *Glossina palpalis* with *Trypanosoma brucei* by allowing the flies to feed for three days upon three animals suffering from "nagana" and then allowed them to feed upon a healthy animal from the fourth day onward. The blood of all the healthy animals bitten was examined frequently and no trypanosomes were found until the twelfth day after the flies had bitten, when an ox and a sheep which had been bitten by flies infected for twelve days, showed the trypanosomes in their blood. In another experiment the flies did not become infective until after a period of twenty days from the time of biting the infected animal. Flies were found to remain infective for from forty to fifty days, and one experiment proved that they remained infective until the sixty-fifth day. Kleine also was successful in infecting laboratory-bred *Glossina palpalis* with *Trypanosoma gambiense* and producing infection in monkeys by the bites of these flies, after a period of several days had passed from the

infective feed of the fly. Kleine also described, in a later paper (1909), the forms of *Trypanosoma gambiense* occurring in the fly, and worked out a life-cycle which he considered to be sexual in character.

Kleine's work concerning the period of non-infectivity of the fly after biting was confirmed by Bruce (1909) in the case of *Trypanosoma gambiense* and *Glossina palpalis*, who found that, in his experiments, the flies became infective 16, 19, and 22 days after biting a sleeping sickness patient.

The work of Kleine and of Bruce has been confirmed by all observers who have studied the subject, and it is now definitely proven that *Trypanosoma gambiense* is transmitted from man to man by *Glossina palpalis*, and that this transmission only occurs, in most instances, after the trypanosome has undergone a cycle of development in the fly. The forms produced during this cycle of development have already been described and it has been noted that the fly does not become infective until the trypanosomes reach the salivary glands and that, after this has occurred, it remains infective until it dies. There is no evidence that infection with *Trypanosoma gambiense* is hereditary in the fly, for the larvæ of infected flies are not infective and do not contain trypanosomes.

Bruce, Hammerton, and Bateman (1910) have shown that only about 1 in every 20 *Glossina palpalis* fed upon infected animals show trypanosomes and become infective. They also demonstrated that in regions in which the infected population has been removed the flies may remain infective for as long as two years, thus suggesting some reservoir of infection for the fly in such regions.

The question of the reservoir of infection for the fly has always been an interesting and important one, for, while in regions in which sleeping sickness is epidemic, infected man is undoubtedly the source of infection of the fly, it is also true that in many regions cases of sleeping sickness are seldom observed and yet fly infection is constantly present. Bruce, Hammerton, and Bateman (1910) found that waterbuck, bushbuck, and reedbuck were all easily infected with a human strain of *Trypanosoma gambiense* by the bites of infected *Glossina palpalis*, and that one exposure to the bites of infected flies was sufficient to cause the infection of these antelope. They determined that the incubation period in the antelope was about seven days, that the infected antelope can convey the infection to clean laboratory-bred *Glossina palpalis* for as long as eighty-one days after infection, and that such infected flies can transmit the trypanosome to susceptible animals by their bites. These observations prove that the antelope may be "potential" reservoirs of infection with *Trypanosoma gambiense*, as these animals are not harmed by the parasite and can harbor it for long periods of time.

Despite a very careful search for naturally infected antelope in regions where sleeping sickness occurs, no natural infection in any species was found until 1912, when Duke found *Trypanosoma gambiense* in the situtunga (*Tragelaphus spekei*), a species of antelope on Damba Island in Victoria Nyanza, and later he found this parasite in the blood of a hyena and buffalo in Uganda. It is not accepted by the German investigators that the trypanosome found by Duke is identical with *Trypanosoma gambiense*, and Kleine, Taute, and others claim that, while the two parasites are indistinguishable morphologically, they are not identical and certainly, in the case of *Trypanosoma rhodesiense* and the trypanosome found in antelope, experiments upon human beings appear to have shown that the parasites are distinct. On the other hand, the ease with which various species of antelope can be experimentally infected by flies infected with *Trypanosoma gambiense* indicates that such transmission can, and, indeed, must occur at times in nature, and while infected man is undoubtedly the chief cause of the transmission of the infection to flies it is, I believe, justifiable to regard some of the game animals of Africa, especially antelope, as reservoirs of infection in certain localities.

Domestic animals may act as a reservoir of infection for flies, as the Royal Society Commission (1910) found a naturally infected cow in an island in Victoria Nyanza, and Kleine and Eckard (1913) found *Trypanosoma gambiense* in a sheep, goat, and ox in German East Africa. Yorke and Blacklock (1915) found the parasite in a cow in Sierra Leone and others have reported finding naturally infected domestic animals. If such infections occur in domestic stock there appears to be no reason why infection should not occur in susceptible game animals.

Transmission of *Trypanosoma gambiense* by coitus has been suggested by some investigators as a common method of transmission. Koch (1907) and Kudicke (1908) give instances of infection with this parasite in women whose husbands were infected and who lived in districts where no *Glossina palpalis* occurred. In one district in German East Africa, Koch discovered the organism in fifteen women who were married to men with sleeping sickness, and the most careful survey of the localities in which these women lived, and had always lived, failed to show any *Glossina palpalis*. That such a method of infection is possible can hardly be denied, in view of experimental evidence that has accumulated, but it is not believed at present that it is a very common method of transmission of the disease. Hindle (1911) failed in his endeavors to transmit *Trypanosoma gambiense* to healthy female rats by allowing them to live with infected males, but he found that infection of the female could be brought about by placing a drop of infected blood in the vagina. He also found that infection could be brought

about in rats by placing a drop of blood containing many *Trypanosoma gambiense* upon the healthy skin.

*Hereditary transmission* of *Trypanosoma gambiense* from mother to child has not been demonstrated and the evidence is conclusive that mothers suffering from sleeping sickness do not transmit the infection to their offspring. Very careful examinations have been made, by numerous observers, of still-born babes of infected mothers, and no one has yet recorded finding *Trypanosoma gambiense* in the blood or tissues of these children.

Although hereditary transmission of infection with this parasite does not occur, it has been proven that the organism may pass into the milk of the infected mother and that the child can be infected in this manner. Lanfranchi (1916) has shown experimentally that *Trypanosoma gambiense* passes into the milk of infected bitches and that the young may be infected by suckling, and his observations have been confirmed by other investigators.

**Experimental Infection of Lower Animals.**—*Trypanosoma gambiense* can be transmitted to most mammals, especially to game animals, domestic animals, and laboratory animals. Infection does not always follow inoculating of virulent material, and in most animals the symptoms produced are slight, fever being the most marked and constant symptom. The animal reactions are chiefly depended upon in the differentiation of *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, the latter species being much more virulent to most animals and the infection produced by it lasting a much shorter time before death occurs.

In animals infected with *Trypanosoma gambiense* trypanolytic substances are produced which act upon the homologous trypanosome, but not upon other trypanosomes, and this reaction is of service in differentiating the species from *Trypanosoma rhodesiense*. It has also been proven that animals immunized against *Trypanosoma gambiense* are not resistant to infection with *Trypanosoma rhodesiense*. Thus, Laveran immunized a goat and mice to *Trypanosoma gambiense* and then inoculated them with *Trypanosoma rhodesiense*, infection with the latter parasite occurring in every instance. These cross-immunity tests are of great service in differentiating species of trypanosomes, and have proven beyond doubt the non-identity of *Trypanosoma gambiense* and *Trypanosoma rhodesiense*.

Agglutinins are produced in the blood serum of animals inoculated with *Trypanosoma gambiense* after a few injections of blood containing the parasite, and if the blood serum of such an animal be added to blood containing the trypanosomes, marked agglutination of the organisms will occur, masses being formed in which the flagellated ends of the parasite project outward.



The virulence of *Trypanosoma gambiense* to the lower animals depends upon the origin of the strain inoculated, the genera and species of animal inoculated, and upon successive passage through certain species of animals.

In *monkeys*, of which species belonging to *Macacus* and *Cercopithecus* are most susceptible, the inoculation of blood containing *Trypanosoma gambiense* is followed, after an incubation period varying from eight to as long as forty days, by slight fever and the symptoms of a typical attack of sleeping sickness, or, more often, by emaciation, anæmia, lowering of the temperature, and a tendency to somnolence. The trypanosomes may be found in the blood and in the cerebrospinal fluid in those animals presenting marked cerebral symptoms. Infection in the monkey is said to be invariably fatal. The higher apes appear to be more resistant to infection with this trypanosome, but Gray and Tulloch (1905), Thomas and Breinl (1905), and others have produced typical sleeping sickness in baboons. The duration of the infection in monkeys is usually several months, from six to seven, as a rule, but some animals live for over a year after infection.

In *dogs*, inoculation with *Trypanosoma gambiense* is followed by a chronic infection varying in duration, but extending over several months, as a rule. The period of incubation and the duration of the infection in dogs is variously given by different investigators, thus indicating that they worked with trypanosomes varying greatly in virulence. The Uganda Commission found the incubation period to be from two to five weeks in dogs, while Brumpt and Wurtz (1904) give the incubation period in these animals as seventeen days. These authors state that the duration of the infection in dogs is only thirty-six days, while Thomas and Linton (1904) give the duration as from three weeks to nine months.

The symptoms produced in dogs by inoculation of *Trypanosoma gambiense* are emaciation, anæmia, attacks of fever, with subnormal temperatures before death. The trypanosomes may be found in the blood in small numbers.

*Trypanosoma gambiense* is also infective to the jackal, cats, rabbits, guinea-pigs, white rats, mice, jerboas, marmots, goats, sheep, and horses. Cattle are resistant, but may be infected. In all of these animals the infection runs a chronic course and does not result fatally in the larger mammals, as sheep, horses, and cattle.

**Relation to Disease.**—It has been proven beyond doubt, both by animal experimentation and by the constant presence of *Trypanosoma gambiense* in the blood and cerebrospinal fluid of patients suffering from one form of sleeping sickness that the parasite is the cause of the disease. As already stated, this species is the cause of sleeping sickness in only

certain localities in Africa, but its distribution is much more extensive than that of *Trypanosoma rhodesiense*, and it is responsible for most of the morbidity from this disease. The parasite has never been found in either the blood or cerebrospinal fluid of patients suffering from any other disease or in healthy individuals, but it is always present in both the blood and cerebrospinal fluid, at some time, in persons suffering from the form of sleeping sickness with which it is associated. The parasite has been cultivated and cultures have produced in susceptible animals the typical symptoms of sleeping sickness as observed in man. The inoculation of the blood or cerebrospinal fluid containing the parasite is followed in susceptible animals by sleeping sickness, and the trypanosomes can be recovered in their blood and cerebrospinal fluid. Finally, it has been proven that *Trypanosoma gambiense* undergoes a definite cycle of development in *Glossina palpalis* and other flies of this genus, and that such flies, after a period of incubation, can, by their bites, produce in susceptible animals the characteristic symptoms of the infection. Today no one doubts the etiological relationship of *Trypanosoma gambiense* to the form of sleeping sickness with which it is associated.

It is not the province of this work to enter into the clinical signs or symptoms of this infection, but it may be stated that the parasite causes an infection characterized by irregular fever, emaciation, cutaneous eruptions, enlargement of the lymphatic glands, severe nervous symptoms, among which lethargy and sleepiness are most marked, and ending finally in coma and death in the vast majority of cases.

The pathology of sleeping sickness is quite characteristic and is most marked in the glandular tissues and nervous system. Macroscopically there is generally a wide-spread enlargement of the lymphatic glands, especially of those situated in the cervical, submaxillary, femoral, and inguinal regions. These enlarged glands may appear much congested and even hæmorrhagic, and in such instances are soft in consistence, but in the more chronic infections the glands may be increased in consistence and little, if any, enlarged. The spleen is enlarged and may appear greatly congested in acute cases. The liver may also be enlarged and congested.

Macroscopically the brain shows the usual evidences of a chronic leptomeningitis. The cerebrospinal fluid is cloudy and increased in quantity and the membranes are thickened in places and adherent. There is no change in the appearance of the brain substance, and but little evidence of congestion.

The microscopical pathology was first accurately described by Mott (1905-1906) and his description has been confirmed by numerous investigators. The essential feature of the infection is a chronic inflammation of the lymphatic apparatus, especially of the lymphatics of the

brain and spinal cord. The base of the brain is especially involved in the process. There is a proliferation of the neuroglia cells, of the endothelial cells, and an invasion of the pia-arachnoid membrane with lymphocytes. A peculiar type of cell occurs in all the lesions, known as the plasma cell of Marschalko, which contains a nucleus having a wheel-like arrangement of the chromatin. These cells, according to Mott, are derived from the lymphocytes.

The most characteristic change noted in the nervous system is the extensive perivascular infiltration around the blood-vessels, the infiltrating cells consisting of proliferated neuroglia cells, lymphocytes, nuclei of endothelial cells, the plasma cells of Marschalko, and large phagocytic cells, or macrophages, which contain red blood corpuscles. The perivascular infiltration resembles that present in syphilis of the nervous system.

Regarding the microscopical pathology of the nervous system in infection with *Trypanosoma gambiense*, Blacklock and Yorke (1922), in an excellent discussion of the subject, state: "The nuclei of the endothelial cells of the capillaries of the pia and brain tissue may show proliferation, and in the neighborhood of the capillaries and small vessels there are often numerous lymphocytes, plasma cells, and glia cells. Capillary hæmorrhages are sometimes met with. As a result of these interstitial lesions there is usually some secondary parenchymatous atrophy. In chronic cases marked chromatolytic changes and atrophy of dendrons may occur in the ganglion cells, especially in those regions where the perivascular infiltration is severe and where, in consequence, a certain amount of blood stasis takes place."

All of the changes briefly described are believed by Mott to be due to the irritation of the tissues by the trypanosomes present in the cerebrospinal fluid, but the more recent work of Yorke (1911), Stephenson (1922), and others have shown that the trypanosomes actually leave the blood-vessels and lymphatics and penetrate into the surrounding tissues, being found within the connective tissue of the organs and in the nervous tissue. As Yorke states, in view of these findings, it is very probable that the lesions of the infection are due to the action of trypanosomes within the tissues.

**Prophylaxis.**—The prophylaxis of infection with *Trypanosoma gambiense* would appear to be a simple matter, as we know the insects that transmit the infection, and their destruction or protection from their bites will prevent infection. However, the practical difficulties connected with the prophylaxis of this infection have proven almost insurmountable in many of the endemic regions, and there is still the greatest difference of opinion among the best authorities as to the relative value of certain prophylactic methods and as to whether some methods that have been employed were justified by the results.

Theoretically, the destruction of the transmitting fly, *Glossina palpalis*, would be the ideal method of prophylaxis, but it has been found impossible to prevent the breeding of these insects in many, if not most, of the endemic centres of the disease. However, a great diminution can be accomplished in the number of these insects by clearing undergrowth and bush, especially around watering-places, fords, landing-places, and areas along rivers or lakes used for washing and laundry purposes. The larvæ of *Glossina palpalis*, like the larvæ of all species of this genus, develop one at a time in the uterus of the fly, and when extruded are practically adult, pupating without taking any further food. After pupation the young fly takes its first food in the form of blood obtained from an animal or man. The distribution of the fly is dependent upon a moist atmosphere and is confined to the banks of rivers, streams, or lakes. Necessary for its development, according to Fiske, are deep shade for shelter, light shade for flight and to protect its breeding-places, sand, gravel, or dry vegetable débris for a breeding-place, and an abundant supply of suitable food, which means the blood of animals or man. All of these requirements are found in the localities mentioned above, and hence the necessity of destroying the vegetation in these places if one expects to destroy the flies. As shown by numerous investigators, the clearing of such areas as those mentioned has resulted in a great diminution in the number of *Glossina palpalis* in certain localities, and in limited districts, where conditions have been very favorable, in their entire disappearance, but, unfortunately, such successful results have been the exception, and fly extermination by clearing has not been of great practical value in most regions in which it has been tried. However, as much as is practicable in this direction should be done, as experience has proven that some results may be expected if the cleared areas are kept cleared continually. The clearing should cover at least two hundred yards on each side of a ford, and the same area should be cleared around landing-places, watering-places, or on the banks of streams where washing is done. It is not necessary to cut down the tall trees that have no branches within fifteen feet of the ground, but all undergrowth and low branches should be cut out and burned.

Trapping of the flies has been extensively tried and has been found a useful prophylactic measure, and the importation of insect, or other, parasites of the fly has been suggested as a possible measure of value.

The *depopulation of districts* in which sleeping sickness is endemic, and epidemics occur, has been advocated by some authorities and has proven very successful in the prophylaxis of the infection. In Uganda, according to Blacklock and Yorke (1922), almost 24,000 persons were removed from the shores of Lake Victoria and the Buvuma and Sesse Islands, owing to the prevalence of this infection, with the consequent



cessation of the epidemic, although these people were only removed for a distance of about two miles. This method of prophylaxis, however, should only be resorted to in the presence of an epidemic owing to the economic questions involved.

The isolation of the infected individual in screened habitations in order to prevent fly infection is most important. Individuals infected with *Trypanosoma gambiense* should be screened, but this measure is often impracticable in the regions in which the infection occurs, although it should be employed whenever possible.

The proper treatment of patients infected with this parasite is a more important prophylactic measure. The inhabitants of regions in which sleeping sickness occurs should be carefully examined and all found infected should be segregated, protected from the bites of tse-tse flies, and given adequate treatment. While a trypanosome indistinguishable from *Trypanosoma gambiense* has been found in antelope, in sleeping sickness districts, by Duke and others, it is generally admitted that man is the chief source of infection of the fly in these regions and, therefore, prophylactic measures directed toward rendering man harmless to the fly are those that will be the most successful. Protection of infected individuals from the bite of *Glossina palpalis*, thus preventing the infection of the fly, and the destruction of the trypanosomes in the blood of the infected individual, are prophylactic methods of the greatest value.

The prophylactic value of the *destruction of the wild game* in regions where sleeping sickness occurs is discussed in considering the prophylaxis of infection with *Trypanosoma rhodesiense*. That certain wild animals act as reservoirs of infection for both *Trypanosoma gambiense* and *Trypanosoma rhodesiense* can hardly be doubted, in view of the evidence that has accumulated, but there is still the greatest difference of opinion among authorities regarding the practical value of the destruction of such animals in the prophylaxis of the infection.

*Personal prophylaxis* against infection with *Trypanosoma gambiense* consists of measures which will protect one from the bite of *Glossina palpalis*. White clothes should be worn, as these insects appear to be attracted by dark clothes. The legs should be protected by puttees or leggings and the head and hands by netting and gloves respectively. In travelling through districts infected with *Trypanosoma gambiense*, it is best to travel by night instead of by day.

The French Sleeping Sickness Commission, composed of Brumpt, Gouzien, Laveran, Lebœuf, G. Martin, L. Martin, Mesnil, and Roubaud (1920), made the following recommendations regarding the prophylaxis of infection with *Trypanosoma gambiense*:

1. Adequate treatment of all individuals showing trypanosomes in their blood or who present symptoms of sleeping sickness. If a thorough

course of treatment cannot be administered they recommend two subcutaneous injections of from 0.75 to 1 gm. of atoxyl at an eight-day interval, repeated every two months or every four to six months. This atoxylization of infected individuals they consider most important in prophylaxis as it rids the peripheral blood of the trypanosomes and thus prevents infection of the fly.

2. The removal of the inhabitants from areas in which *Glossina palpalis* is present.

3. The protection of man from the bites of *Glossina palpalis*.

4. The proper control of individuals travelling from infected to clean areas.

5. Clearing and destruction of the breeding-places of *Glossina palpalis*.

As already noted, the destruction of game animals, especially the antelope, has been urged as a prophylactic method of value, but, in the case of *Trypanosoma gambiense*, it is doubtful if much would be accomplished by the destruction of game, as man is the chief source of infection to the fly. The destruction of the game would, of course, remove a very important source of food supply for the flies, which feed upon these animals, but it would be necessary not only to destroy the antelope, but also the crocodiles and monitor lizards upon which *Glossina palpalis* feeds habitually, and this would be impossible in many regions where sleeping sickness is endemic. It will be noted that the French Commission did not include game destruction in their recommendations regarding the prophylaxis of the disease.

Species II. *TRYPANOSOMA RHODESIENSE*, Stephens and Fantham, 1910.

**History and Nomenclature.**—In 1909, Stephens and Fantham found a trypanosome in the blood of an Englishman suffering from symptoms suggestive of sleeping sickness in Northeastern Rhodesia, a region in which *Glossina palpalis* does not occur. This trypanosome they regarded as a new species and named it *Trypanosoma rhodesiense*. They published their discovery in 1910, and gave a detailed description of the trypanosome. In 1912, Kinghorn and Yorke proved that *Trypanosoma rhodesiense* is transmitted from man to man by a tse-tse fly, *Glossina morsitans*, the same species concerned in the transmission of *Trypanosoma brucei*, the cause of "nagana" in domestic stock. These investigators also proved that *Trypanosoma rhodesiense* undergoes a definite cycle of development in *Glossina morsitans* similar to that of *Trypanosoma gambiense* in *Glossina palpalis*.

That *Trypanosoma rhodesiense* is a valid species is now accepted by the majority of protozoologists, but there are some authorities who believe that it is identical with *Trypanosoma brucei*. In my opinion, the

experiments upon man by Taute, hereafter mentioned, furnish conclusive evidence that *Trypanosoma rhodesiense* and *Trypanosoma brucei* are not identical, but are distinct species.

**Morphology in Man.**—In the blood of man the morphology of *Trypanosoma rhodesiense* is so similar to that of *Trypanosoma gambiense* that the two parasites are practically indistinguishable. However, in the blood of experimentally infected animals the so-called "posterior nuclear forms" are found, in which the nucleus or trophonucleus is situated near the posterior, or non-flagellate, extremity of the para-



FIG. 53.—*Trypanosoma rhodesiense*. 1. Long narrow form. 2. Intermediate form. 3, 4 and 5. Short broad forms. In 3, 4 and 5 the nucleus is shown passing to the posterior (non-flagellate) end of the trypanosome. X 1,800. (After Stephens and Fantham.)

site, close to or even behind the blepharoplast. Such posterior nuclear forms are never observed in experimental animals in the case of *Trypanosoma gambiense*, but are observed in infections with *Trypanosoma brucei*. When the nucleus is in this situation it is kidney-shaped and the trypanosome is short and broad. Not all short, broad forms have posterior nuclei, however, for Stephens and Fantham found that only about six per cent. of such forms had the nucleus displaced backward. The same variations occur in the form and size of this species in the blood of man as are noted in infections with *Trypanosoma gambiense*, long, slender forms, short, broad forms, and forms intermediate in morphology being found in the peripheral blood.

Until recently, the posterior nuclear forms of *Trypanosoma rhodesiense* were never found in the blood of infected individuals, but Mackenzie (1922) found posterior nuclear forms in the peripheral blood of a fatal case of sleeping sickness in a European woman. The failure to find them before has been due to the fact that this species of trypanosome occurs in very small numbers in the peripheral blood of most cases of infection with this parasite, and the finding of Mackenzie must be regarded as altogether exceptional and one which will probably not be repeated for some time.

Metachromatic granules are frequently observed in the cytoplasm

of the flagellate, or anterior, extremity of the organism, and such granular forms have been considered as specific, but the same granulation may be observed in certain strains of *Trypanosoma gambiense*.

The length of *Trypanosoma rhodesiense* is given by Stephens and Fantham as from 12 to 39 microns, the short, broad forms measuring from 13 to 21 microns, the intermediate forms from 21 to 24 microns in length, and the long, slender forms from 25 to 39 microns. The average length is about 24.1 microns.

As in infections with *Trypanosoma gambiense*, periods occur in which

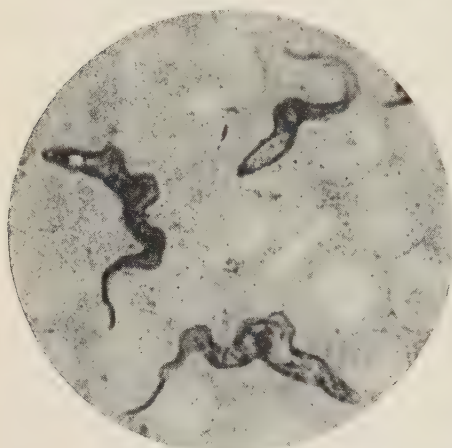


FIG. 54.—*Trypanosoma rhodesiense*.  $\times 1,400$ . (Photomicrograph. Army Medical School Collection.) Wright's stain.

the peripheral blood contains very few parasites or is apparently free from them, and at such times "latent forms" occur in the internal organs, especially in the spleen and lungs. These forms are similar in morphology to the latent forms found in infections with *Trypanosoma gambiense*, first observed by Moore and Breinl (1907), and they have been thoroughly studied and described by Fantham (1911), who observed the development of these forms from the typical motile forms of *Trypanosoma rhodesiense*, and

their later reversion to the typical motile forms when conditions became favorable. The morphology of these forms has already been described in the discussion of the morphology of *Trypanosoma gambiense*.

It should be remembered that a differential diagnosis cannot be made between *Trypanosoma rhodesiense* and *Trypanosoma gambiense* by comparing the forms found in the peripheral blood of man. Such a diagnosis must rest upon the finding of the peculiar post-nuclear forms found in the blood of an inoculated animal.

**Morphology in *Glossina Morsitans*.**—The morphology of *Trypanosoma rhodesiense* during its development within the alimentary tract and salivary glands of *Glossina morsitans* is practically the same as already described for similar stages in the life-cycle of *Trypanosoma gambiense* in *Glossina palpalis*.

The staining reactions of *Trypanosoma rhodesiense* are identical with those of *Trypanosoma gambiense*, the cytoplasm staining a blue or violet with the Wright stain, the trophonucleus a pink or rose-violet, the



blepharoplast dark red or almost black, the undulating membrane blue, the border being formed by the pink or dark red stained flagellum.

**Habitat.**—*Trypanosoma rhodesiense* lives in the blood and cerebrospinal fluid of man and, according to Blacklock and Yorke, Bruce, and others, in the blood of game animals, especially various species of antelope.

**Species Occurring in Lower Animals.**—The most important species of trypanosomes occurring in lower animals have already been mentioned (see page 233), and are briefly described later, but *Trypanosoma rhodesiense* has been reported by Kinghorn and Yorke (1912) as occurring in 16 per cent. of the game animals in the Luangwa Valley, Northern Rhodesia, and 3.3 per cent. on the Congo-Zambezi watershed. The German investigators, while admitting that a trypanosome very similar in morphology and pathogenicity occurs in the game in regions where *Trypanosoma rhodesiense* is endemic, do not believe the game trypanosome to be identical with *Trypanosoma rhodesiense*, and the experiments of Taute (1919) upon infection of man with the game trypanosome, in which over 100 individuals were inoculated with this organism without an infection occurring, offer conclusive proof of the non-identity of the game trypanosome with *Trypanosoma rhodesiense*.

**Cultivation.**—The cultivation of *Trypanosoma rhodesiense* is difficult, but has been successfully accomplished by Thompson and Sinton (1912) on a modified N.N.N. medium. They succeeded in cultivating the parasite for 21 days at a temperature between 22–24° C. The forms occurring in the cultures were similar to those found in the alimentary tract of *Glossina morsitans* and to those of *Trypanosoma gambiense* in cultures.

**Life-history.**—The life-history of *Trypanosoma rhodesiense*, both in man and in *Glossina morsitans*, so far as it has been determined, is exactly like that of *Trypanosoma gambiense*. In man multiplication occurs by longitudinal division, the process being initiated by the division of the blepharoplast, as in *Trypanosoma gambiense*. After a period of longitudinal division in the peripheral blood the parasites become quiescent and the so-called latent forms develop in the internal organs, especially in the lungs and spleen. When conditions again become favorable the latent forms develop into typical flagellates and multiplication again occurs by longitudinal division.

The life-cycle in the fly is similar to that of *Trypanosoma gambiense* in *Glossina palpalis*. The fly does not become infective, according to Kinghorn and Yorke (1913), for a period of from 10 to 25 days after feeding upon blood containing the trypanosomes. The developmental forms found in the intestine and salivary glands of *Glossina morsitans* do not differ in their morphology from those already described as occurring in flies infected with *Trypanosoma gambiense*, the long, slender

forms being most numerous in the intestine and proventriculus. These forms invade the salivary glands, where crithidial and encysted forms develop from them, and later these forms develop short, broad forms which are infective to man. *Glossina morsitans* does not become infective to man until the trypanosomes invade the salivary glands, but after this has occurred, even the forms in the intestine are infective.

**Geographical Distribution.**—The geographical distribution of *Trypanosoma rhodesiense* is much more limited than that of *Trypanosoma gambiense*. Both species are limited in their distribution to Africa, but *Trypanosoma rhodesiense* has been found in comparatively limited districts of this continent. This species has been found in Northeastern Rhodesia, in the northern part of South Rhodesia, in Nyassaland, in German and Portuguese East Africa, and recently Archibald (1922) has reported one case of infection with this trypanosome in the Bahr-el-Ghazal region of the Sudan. The infection has been found especially prevalent in the Luangwa Valley, in Northeastern Rhodesia, and in the Rovuma Valley, in German East Africa.

**Incidence of Infection.**—Unlike infections with *Trypanosoma gambiense*, infections with *Trypanosoma rhodesiense* are not numerous, even in the endemic regions, and epidemics of sleeping sickness due to this trypanosome have never been reported. Why this is so is still unsolved, although there are several theories which endeavor to explain it. Those who believe that *Trypanosoma rhodesiense* is identical with *Trypanosoma brucei* explain the relatively slight incidence of the infection in man by stating that *Trypanosoma brucei*, being a parasite of lower animals, has not yet become adapted to life in the human host, and only a comparatively few individuals are susceptible to infection with this trypanosome. However, it is very doubtful if these species are identical, so that this explanation, plausible as it may sound, is probably erroneous.

**Method of Transmission.**—*Trypanosoma rhodesiense* is transmitted to man by *Glossina morsitans*. While in nature this species of *Glossina* is the carrier of the infection, it has been proven that other species of *Glossina* may be experimentally infected with this trypanosome, and that it undergoes the same cycle of development in such flies. Eckard (1913) proved that *Trypanosoma rhodesiense* can develop in *Glossina palpalis*, and the Royal Society Commission (1914), that it can develop in *Glossina brevipalpis*. Duke (1923) has recently described an outbreak of sleeping sickness due to *Trypanosoma rhodesiense*, in which the trypanosome was transmitted by a new species of *Glossina*, *Glossina swinertoni*, Austen, 1923. Further investigation will probably show that while certain species of *Glossina* are more favorable to the development of the trypanosomes infecting man than others, development can occur in a number, if not all, species. Why development occurs in na-

ture in only one species has not been ascertained, but it is certain that each of the trypanosomes of man, for all practical purposes, is confined in its development to a single species of *Glossina*.

Other insects, as mosquitoes, ticks, bed-bugs, and lice, have been suggested as possible vectors of the trypanosome, and while mechanical transmission might occur by these insects, it is not probable that any insects other than flies act as transmitters of the infection.

*Glossina morsitans* may transmit *Trypanosoma rhodesiense* to man either mechanically or after a developmental cycle has been passed in the fly by the trypanosome.

*Mechanical transmission* is not believed to be very important in nature, but undoubtedly may occur. It has been determined by Yorke, Bruce, and others, that for mechanical transmission to occur, the fly must bite within a few hours after feeding on an infected individual, and that it is impossible for the insect to transmit the infection mechanically after twenty-four hours.

In the vast majority of cases infection with *Trypanosoma rhodesiense* is transmitted *after a period of development* has occurred within the alimentary tract and salivary glands of *Glossina morsitans*. The development within the fly is controlled very largely by the temperature to which the insect is exposed. Kinghorn and Yorke (1913) found that multiplication of the trypanosome occurred within the intestine of the fly at temperatures between 55° and 65° F., but no infection of the salivary glands occurred unless the temperature was at least 75° F., and that the fly did not become infective during the cold season. This fact explains why infective flies are so much more common in some localities than in others, where other conditions are equal. Thus Kinghorn and Yorke found that the percentage of infective flies was 1-534 in hot valley regions having temperatures ranging from 75° to 85° F., while in cool plateau regions, where the temperature ranged from 60° to 70° F., the percentage of flies that were infective was only 1-1,312. In the Luangwa Valley, where the temperature was high, these investigators found *Trypanosoma rhodesiense* in 7 of 3,202 flies examined, while at a place only 78 miles distant, where the temperature was much lower, they found only 4 of 5,250 *Glossina morsitans* infected with *Trypanosoma rhodesiense*.

As will be noted, only a small proportion of the flies examined, even where temperature conditions are most favorable, show infections with the parasite. Bruce and his co-workers (1914) found that only 8 per cent. of *Glossina morsitans*, when allowed to feed upon patients infected with *Trypanosoma rhodesiense*, became infected, and that only about 1 per cent. of these flies became infective.

The period of incubation in the fly, that is, the time elapsing between the infective feed of the fly and the time that the insect be-



comes infective, was shown by Kinghorn and Yorke (1912) to vary between 10 and 25 days, and Bruce and his colleagues, in their experimental work, found that it varied between 14 and 31 days at a temperature of 84° F. The infection in the fly is not hereditary.

As already stated, the morphology of *Trypanosoma rhodesiense* during its development in *Glossina morsitans* is similar to that of *Trypanosoma gambiense* in *Glossina palpalis*.

**Reservoirs of Infection and the Identity of *Trypanosoma rhodesiense* and *Trypanosoma brucei*.**—Infections with *Trypanosoma rhodesiense* are generally few in number, even in endemic centres of the disease, and epidemics like those caused by *Trypanosoma gambiense* have never been reported. The sporadic occurrence of this infection, the cases often being widely separated in point of distance, has led to the assumption that one or more of the lower animals must act as a reservoir of the infection, as otherwise it is difficult to explain why the infection does not disappear. Kinghorn and Yorke (1912) examined the wild game in regions where *Trypanosoma rhodesiense* occurred and found about 16 per cent. naturally infected with a trypanosome indistinguishable from *Trypanosoma rhodesiense*. They examined water-buck, bushbuck, hartebeest, impala, and wart-hogs, and found instances of infection in all of these animals. The fact that inoculation of these animals with *Trypanosoma rhodesiense* is not followed by any appreciable symptoms, although their blood may contain the parasite and be infective to flies, and that the animals found naturally infected with an identical parasite, morphologically, show no clinical signs of the infection, is considered proof, by those who believe that the wild game act as reservoirs of infection for *Glossina morsitans*, of the truth of their contention. Duke (1915) instances the cases of two of his native "fly" boys who developed infection with *Trypanosoma gambiense* from the bite of flies in a region where the infected population had been removed five years previously, proving, in his opinion, that the parasite still was present in this region and must have been perpetuated in the wild game.

The German investigators, as Kleine, Taute, and Fischer, do not believe that the trypanosome found in the wild game in regions where *Trypanosoma rhodesiense* occurs is identical with the latter species, although they admit that a considerable proportion of the game is infected with a trypanosome practically indistinguishable from *Trypanosoma rhodesiense* in morphology and in its effects upon susceptible animals.

It is now generally admitted that the trypanosome found in the wild game by Kinghorn and Yorke is identical with *Trypanosoma brucei*, so that the question at issue is whether *Trypanosoma rhodesiense* is identical with *Trypanosoma brucei*, which is found not only in the wild game,



but in domestic stock in regions where sleeping sickness, due to *Trypanosoma rhodesiense*, is endemic.

Kinghorn and Yorke (1912) and Blacklock and Yorke (1922) believe that the two species mentioned above are identical and that the sporadic occurrence of the infection in human beings is due to a natural immunity against *Trypanosoma rhodesiense*. They believe that the trypanosome exists endemically in game and the fly throughout the country, and that only those individuals whose natural resistance or immunity to the parasite is lowered, or absent, develop the infection. They point to the fact that the antelope are susceptible to infection with *Trypanosoma rhodesiense* experimentally, by the bite of infected *Glossina morsitans*, and that these animals must become infected naturally in regions where *Trypanosoma rhodesiense* occurs.

Taute and the German investigators deny that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are the same species for the following reasons: *Trypanosoma brucei* is found both in wild game and in domestic animals in regions where *Glossina morsitans* is present, but where no cases of human infection have ever been encountered; that Europeans live in these regions without contracting the infection; that cattle and dogs die of infection with *Trypanosoma brucei* where infection of man is unknown; that during the World War, thousands of troops operated in the districts infected with *Trypanosoma rhodesiense* without a single case of infection of man occurring until February, 1917, and that the total number of cases that occurred was less than half a dozen; that during this period the transport animals suffered greatly from infection with *Trypanosoma brucei* and conditions were most favorable for transmission to man, if such transmission could occur, but no infections resulted; and finally, that the actual inoculation of man with *Trypanosoma brucei* does not result in infection and the occurrence of symptoms of sleeping sickness.

Taute (1919) inoculated himself and ten natives with *Trypanosoma brucei* obtained from a naturally infected mule suffering from "nagana," five of the natives being in good health and five in poor condition, due to other infections, as malaria, hook-worm, etc. The most careful examinations proved that no infection occurred in any of the individuals inoculated. Not content with these results, which most investigators would have considered conclusive, Taute and Huber (1919) inoculated themselves and 129 natives with *Trypanosoma brucei* from four naturally infected horses and two mules. Many of the natives were in poor health due to repeated attacks of malaria and other infections, and others were in robust health. In no instance was an infection produced by the inoculations, although control animals developed the infection.

These experiments of Taute and Huber are sufficient, I believe, to

prove that *Trypanosoma brucei* is not identical with *Trypanosoma rhodesiense*, and that it is not capable of producing sleeping sickness in man. Blacklock and Yorke (1922) still adhere to their belief that man is very resistant to infection with *Trypanosoma rhodesiense* (*Trypanosoma brucei*) and that this resistance explains Taute and Huber's results. It seems almost too much of a strain on one's credulity to believe that of one hundred and thirty-one inoculated individuals there was not one susceptible to infection with this parasite, which must have been the case if natural immunity is to explain their results. If this is so, there is no parallel instance, in our knowledge of immunity, in which the inoculation of 131 individuals with a known pathogenic parasite was not followed by a single infection. I believe that the evidence is amply sufficient to prove that *Trypanosoma brucei*, or the trypanosome inoculated by Taute and Huber, is not pathogenic to man and that it is not identical with *Trypanosoma rhodesiense*.

However, the experiments of Taute and Huber do not prove that *Trypanosoma rhodesiense* is never transferred by *Glossina morsitans* from man to the antelope in nature, and it may well be that these animals do act as a reservoir of infection, as it is impossible to differentiate *Trypanosoma rhodesiense* and *Trypanosoma brucei* in infected animals. Taute and Huber performed their inoculation experiments on man in a region known to be free from sleeping sickness, and undoubtedly worked with *Trypanosoma brucei* and, in the opinion of most authorities, have proven conclusively that this trypanosome is harmless to man, but if their work had been done in a sleeping sickness region their results might have been quite different, as some of the antelope in such a region might have been infected with *Trypanosoma rhodesiense*. It must be admitted that the question of the relation of wild game to the transmission of sleeping sickness in the regions in which this infection occurs is still unsolved despite the experiments of Taute and Huber.

*Hereditary transmission* of *Trypanosoma rhodesiense* in man has never been observed, but Bassett-Smith (1919) and Stephenson (1919) observed this parasite in the placental blood of an infected rat and in blood obtained from the liver of the embryos. Lanfranchi (1916) demonstrated that the parasite passes into the milk of infected animals and that the milk is infective, and his observations have been confirmed by others.

**Experimental Infection of Lower Animals.**—Domestic and laboratory animals can be infected with *Trypanosoma rhodesiense* with ease, and the infection produced is rapidly fatal in most instances. Fantham and Thompson (1911) proved that this species of trypanosome is much more virulent to experimental animals than *Trypanosoma gambiense*, and this fact, together with the appearance of the posterior nuclear

forms in the blood of the inoculated animals, is largely depended upon in the differentiation of the two species. Thus, in goats and sheep, *Trypanosoma rhodesiense* causes a rapidly fatal disease, while *Trypanosoma gambiense* causes a long-continued infection in which fever is practically the only symptom. The same is true of infection with this parasite in the small laboratory animals, as the rat, guinea-pig, and rabbit, infection with *Trypanosoma gambiense* generally lasting for several months before death occurs, while infection with *Trypanosoma rhodesiense* seldom lasts for more than a month before the fatal outcome, and frequently for less than twenty days.

As already stated, Laveran immunized animals against *Trypanosoma gambiense* and found that they were still susceptible to infection with *Trypanosoma rhodesiense*. Owing to the great difficulty of immunizing animals to *Trypanosoma rhodesiense*, on account of its virulence, little has been accomplished in proving the reverse of this phenomenon, but Laveran and Nattan-Larrier did succeed in immunizing a ram against *Trypanosoma brucei* and found that it was not immune to *Trypanosoma rhodesiense*, and Laveran immunized both a ram and a sheep against *Trypanosoma brucei*, and afterward produced a fatal infection in both animals with *Trypanosoma rhodesiense*. These experiments prove that *Trypanosoma brucei* and *Trypanosoma rhodesiense* are not identical, but are distinct species.

In animals inoculated with *Trypanosoma rhodesiense*, immune bodies can be demonstrated in small amount in the blood serum. Agglutinins and trypanolytic substances have been claimed to occur by some observers, but in such small amounts as to be useless either in diagnostic or immunity reactions.

While *Trypanosoma rhodesiense* almost invariably produces a fatal disease when inoculated into domestic or laboratory animals, the inoculation of this parasite into wild game, as antelope, is not followed by any symptoms of infection, and animals so inoculated have been observed for long periods of time without any symptoms referable to the infection being noted. This freedom from symptoms after inoculation of a parasite so virulent to domestic and laboratory animals is evidence of a strong immunity in the wild game, and it is certainly suggestive from the standpoint of these animals serving as a reservoir of infection for *Glossina morsitans*.

**Relation to Disease.**—*Trypanosoma rhodesiense* is the cause of the variety of sleeping sickness with which it is invariably associated, a form which differs from the disease produced by *Trypanosoma gambiense* by its more rapid course and the absence of long-continued symptoms of somnolence and of severe involvement of the nervous system. The duration of the disease produced by *Trypanosoma rhodesiense* is

measured by months, while that produced by *Trypanosoma gambiense* usually lasts for several years. Infections with *Trypanosoma rhodesiense* are also more resistant to treatment.

The incubation period is stated by most authorities to be between ten and fourteen days, while the incubation period in infections with *Trypanosoma gambiense* is generally believed to be much longer, although some authorities maintain that the incubation period in the latter infection is practically the same as in infection with *Trypanosoma rhodesiense*.

The pathology of infections with *Trypanosoma rhodesiense* is the same as that of infections with *Trypanosoma gambiense* and has already been described.

**Prophylaxis.**—The prophylactic measures recommended against infection with *Trypanosoma rhodesiense* are similar to those which have been found useful in the prophylaxis of *Trypanosoma gambiense*, consisting of the destruction of the insect vector, *Glossina morsitans*; protection from the bite of this fly; and isolation and proper control of infected individuals.

The measures to be adopted in controlling the breeding of *Glossina morsitans* differ from those used in controlling the breeding of *Glossina palpalis*, owing to the difference in the breeding-places and habits of this insect. *Glossina morsitans*, unlike *Glossina palpalis*, does not live and breed along the banks of rivers and lakes, and in dark, moist places, but inhabits dry forests having much undergrowth; dry plains; rocky country thinly forested; and, in general, is not dependent upon moisture for development, as is *Glossina palpalis*. It bites all day long and often at night, and will follow men and animals for long distances. This fly will feed upon any vertebrate, but in nature there is no doubt that its chief source of food is the wild game, especially antelope, in the regions in which it is found. Lloyd and Patton (1921) state that the breeding-places of this fly are in the hollows of trees, "under fallen trees slightly raised, in the burrows of animals, in holes in termite mounds, under overhanging rocks, among shingle in the little crevices which form the commencement of flood streams, and in large numbers in the sandy beds of dry streams with overhanging banks. The only point in which all these places agree, is that over and above each is some relatively dark spot in which the pregnant fly can hide."

In order to prevent the breeding of *Glossina morsitans* it is necessary to clear areas around encampments and villages for a distance of several hundred yards and to keep these areas cleared. The villages should be built in a compact manner and kept as free from shade as possible. The expense involved in clearing operations is often so great that the method becomes impracticable, and the most that can be done is to take



such measures to prevent man from the bites of *Glossina morsitans* as are possible.

The question of the destruction of the game animals in regions where sleeping sickness due to *Trypanosoma rhodesiense* occurs, in order to prevent the infection of the fly, is most important, from a prophylactic standpoint, and one upon which the greatest authorities disagree. It has never been tried on a sufficiently large scale to prove whether it would result in either the disappearance of the infection or of the fly, but it would seem that the method is worthy of a thorough trial. Even if the wild game do not act as a reservoir of infection for the fly, and hence, indirectly, for man, it is true that the flies depend largely upon these animals for their food, and the destruction of the wild game might, conceivably, be followed by a great diminution in the number of the flies or perhaps by their disappearance in course of time.

As in infections with *Trypanosoma gambiense*, the protection of man from the bites of the fly, the screening of infected individuals, and their proper treatment, in order to render their blood non-infective, and the control of individuals travelling from infected to clean localities, are all important methods of prophylaxis.

#### THE DIAGNOSIS OF INFECTION WITH *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE*

The early diagnosis of infection with *Trypanosoma gambiense* and *Trypanosoma rhodesiense* is of the greatest importance, as early treatment is the only hope for individuals infected with these parasites. In sleeping sickness regions the occurrence of fever, which is resistant to quinine, accompanied by gland enlargement, is very suspicious and should at once lead to laboratory examinations for the purpose of demonstrating the parasites. With modern methods, carefully applied, it is possible to find the trypanosomes either in the peripheral blood, gland juice, or the cerebrospinal fluid in the vast majority of cases, and in all cases if sufficient patience be exercised in the examinations.

**Examination of the Blood.**—As already stated, both *Trypanosoma gambiense* and *Trypanosoma rhodesiense* occur irregularly in the peripheral blood, sometimes being demonstrated without difficulty by microscopical examination, and sometimes being apparently absent or in such small numbers that ordinary examinations do not reveal the parasites. Even when present, both trypanosomes often occur in small numbers, and several blood smears should be examined before the search is abandoned.

For diagnostic purposes it is not necessary to stain blood smears, as the trypanosomes are most easily detected in fresh specimens by rea-

son of their active movements. All that is necessary is to place a drop of blood upon a slide, cover it with a cover-glass and examine it at once with a 4 mm. ( $1/6$  in.) objective. The trypanosomes may be seen actively wriggling among the erythrocytes, and are best seen after the preparation has been allowed to stand for a few moments, as at first the movement may be so rapid that it is very difficult to distinguish the organisms. An immersion lens may be used for distinguishing structural details, but is not necessary in routine diagnostic work.

If it is desired to prepare permanent specimens, blood smears should be made upon clean cover-glasses or slides and air-dried. For staining any of the modifications of the Romanowsky stain are useful, but in my hands the Wright stain has given perfectly satisfactory results. The Leishman and Hasting's stains are also excellent for staining trypanosomes. The method of using Wright's stain is as follows:

Well-spread blood smears are prepared and allowed to dry in the air. The undiluted Wright stain is then added to the blood smear and allowed to fix the smear for one or two minutes. Distilled water is then added, drop by drop, until a well-marked metallic scum appears upon the surface of the solution. The specimen is now allowed to stain for from five to fifteen minutes, according to the intensity of the stain desired, and the smears are then thoroughly washed in distilled water, dried in the air, and examined without a cover-glass. If it is desired to use a cover-glass, only neutral Canada balsam should be employed. For the examination of stained smears the oil immersion lens should always be used.

In well-stained preparations with the Wright stain the cytoplasm of the trypanosomes should be colored blue or violet, the trophonucleus a pink, crimson, or dark red, the blepharoplast a dark red or deep violet, and the flagellum, from its origin at the blepharoplast along the edge of the undulating membrane and throughout its free portion, a dark red or violet. Practice is required to secure specimens that show all the details of structure, and it should be remembered that the trypanosomes in the cerebrospinal fluid and in the gland juice do not stain so easily or so well as those in the peripheral blood.

Owing to the scarcity of the trypanosomes in the peripheral blood, in many infections, the centrifugation of the blood is advised in all cases in which the examination of the peripheral blood gives negative results. For this purpose 10 c.c. of blood is taken from a vein in the arm, the method of collecting the blood being as follows:

The arm should be thoroughly cleaned and the site of the puncture of the vein brushed with iodine. A sterilized glass syringe of 10 c.c. capacity is used and 1 c.c. of 6 per cent. sodium citrate solution is aspirated into the barrel of the syringe. The syringe, with the needle attached, is held almost parallel to the arm, and the needle quickly and firmly pushed through the skin and directly into the vein selected for puncture. The piston should now be withdrawn slowly until the barrel of the syringe

is filled with blood, when the needle should be withdrawn from the vein and the puncture wound covered at once with a piece of sterile gauze.

After collecting the blood it should be centrifuged at once, and various methods have been recommended. It has been found that Broden's (1920) method, which follows, appears to give the best results as regards the number of trypanosomes obtained after centrifugation.

The blood from the syringe is discharged into a centrifuge tube which should be large enough to contain the entire 10 c.c. and centrifuged at from 900 to 1,000 revolutions per minute for three minutes; the supernatant fluid is then removed and this is centrifuged again at about 1,500 revolutions per minute for 10 minutes; the supernatant fluid is again removed and centrifuged at from 1,800 to 2,000 revolutions per minute for 15 or 20 minutes, and the *deposit* examined for trypanosomes. Smears should be made from the deposit and stained as directed for blood examinations. It will be found that the deposit contains practically all of the trypanosomes.

The diagnostic value of blood examinations for *Trypanosoma gambiense* and *Trypanosoma rhodesiense* has been greatly increased by the use of the centrifuge, as the examination of the peripheral blood in the usual manner is often negative. The percentage of positive results obtained by the examination of blood varies with the stage of the infection and the evolution of the trypanosomes. Ross and Thomas (1911) were the first to demonstrate that the parasites occur in greater numbers in the peripheral blood at one time than at another, and in the case that they studied with reference to this phenomenon, there occurred no less than nineteen rises in the number of the trypanosomes, there being an average of six and a half days between each rise, the shortest period between the rises being four days and the longest eight days. During the rises the temperature became elevated and the enlarged glands painful. The rise was due to the rapid multiplication of the trypanosomes, division of these parasites occurring three to four times within twenty-four hours. During the period between the rises the trypanosomes presented the morphology of the latent or resting forms described by Fantham. The increase in the number of trypanosomes in the peripheral blood does not appear to depend upon a regular cyclical development of the organisms, but rather may be considered as evidence of a struggle between the defensive mechanism of the human body and the aggressive power of the parasite. The observations of Ross and Thomas have been confirmed by many investigators and it follows, therefore, that the percentage of positive findings in the blood varies greatly, as given by different authorities, owing to the fact that the blood has been examined at various times and, in many instances, when the trypanosomes were either absent or so few in number as to be overlooked.

Dutton and Todd (1906) examined the blood of 250 cases of sleeping sickness with the following results: Of 220 cases in which blood



examinations were made directly, without centrifugation, 30 were positive for trypanosomes, or 13.6 per cent. Of 17 cases in which the blood was centrifuged, 8 were positive, or 47 per cent.

Martin and Lebœuf (1908) examined the blood directly in 217 cases of sleeping sickness and found the trypanosomes in 37 per cent. of the cases, while centrifuged blood in 75 cases showed 92 per cent. positive for the trypanosomes. In a later series of examinations these observers record a positive percentage of 36.5 per cent. from direct examination of the blood. Adding together the results of direct examination and centrifugation, they obtained a positive result of 96.8 per cent. in the 517 cases that they examined and, accordingly, these observers regard the examination of the blood as the most valuable of all diagnostic methods. Clapier (1921) examined 93 cases by direct blood examination and found trypanosomes in 19, or 20 per cent. Hechenroth found parasites in the blood by direct examination in 32 per cent. of the cases he examined, and Broden (1920), in the examination of 336 patients, using blood centrifugation, obtained positive results in 80.7 per cent.

It will be noted that centrifuging the blood adds greatly to the chances of finding the trypanosomes, and this method should always be followed if direct examination of the blood gives negative results.

In examining the blood of patients suffering from infection with *Trypanosoma gambiense* and *Trypanosoma rhodesiense* it is often noted that agglutination of the red blood corpuscles is present, and this auto-agglutination of the erythrocytes is considered as a valuable diagnostic sign of infection with these parasites. If present, even in the absence of parasites, it is very suspicious and the patients should be very carefully examined before a negative report is returned as to the presence of sleeping sickness.

**Examination of Glandular Juice. Gland Puncture.**—Greig and Gray (1905) were the first to demonstrate that the juice of the lymphatic glands in human trypanosomiasis contained the trypanosomes in comparatively large numbers, and this discovery has proven of the greatest value in diagnosis. Following a suggestion made by Mott, these investigators examined the contents of the lymphatic glands in fifteen cases of sleeping sickness and found the trypanosomes in all of them, and also found that the organisms were much more numerous than in either the blood or cerebrospinal fluid. They were also able to find the trypanosomes in the gland juice of individuals suffering from the symptoms of the early stage of infection, before the typical symptoms of sleeping sickness had developed, thus demonstrating the value of this method of examination in the early diagnosis of the infection. At first they excised the glands examined, but later found that this was not



necessary, as puncture of the gland and the examination of the drop of juice so obtained gave excellent results.

The following procedure for gland puncture gives good results:

The enlarged gland is selected for puncture and the skin over it is painted with iodine. A sterile, dry glass syringe, with a sharp needle of medium bore, is used. The gland is steadied by one hand while the needle of the syringe is pushed into it with the other, the piston being pushed in. Slow and steady aspiration is now made, the piston being pulled out steadily until about half-way up the barrel of the syringe, when it is allowed to return about half the distance and the needle removed from the gland. The drop of gland juice so secured should be ejected quickly upon a microscopic slide, covered with a cover-glass, ringed with vaseline, and examined at once with a 4-mm. ( $1/6$  inch) objective.

The observations of Greig and Gray and others have shown that gland puncture is one of the best methods of demonstrating the trypanosomes in sleeping sickness and especially in the early cases of infection, when the parasites are very scanty in the blood and entirely absent from the cerebrospinal fluid. When blood examination gives negative results gland puncture should always be resorted to, if possible. The posterior cervical glands have given the best results, the axillary, femoral, and epitrochlear following in order. Unfortunately, enlarged glands are not always present and, in the later stages of the disease, are often atrophic and so hard that gland puncture cannot be performed.

If stained preparations of trypanosomes obtained by gland puncture are desired, it should be remembered that the parasites do not stain so well or so easily as those occurring in the peripheral blood.

The percentage of positive results obtained by gland puncture has varied in the hands of different observers. Martin, Lebœuf, and Roubaud (1908) examined 400 infected individuals and found trypanosomes in the glandular juice of 353, or 88.25 per cent. The cervical glands showed trypanosomes in 71.79 per cent. and the inguinal glands in 66.66 per cent., while in 12 per cent. of 459 cases, gland puncture was impossible, either because the glands were too small or did not exist. Broden (1920) examined 336 patients suffering from sleeping sickness and found the gland juice positive in 87.7 per cent.

In contrast to the large percentage of positive results obtained by the authorities quoted are those of Kinghorn and Montgomery (1909), who obtained only 22 positive results in the examination of 4,934 natives who showed enlarged post-cervical glands. Their results indicate that this method of diagnosis in general survey work would hardly be worth the time expended, but in regions where sleeping sickness is epidemic, gland puncture should always be employed if blood examinations are negative.

**Examination of the Cerebrospinal Fluid.**—It will be remembered that Castellani first found *Trypanosoma gambiense* in the cerebrospinal fluid of natives suffering from sleeping sickness, and the microscopic examination of this fluid is still the most valuable diagnostic measure

we possess in those cases of infection presenting symptoms of involvement of the central nervous system. However, the method is useless unless such symptoms are present, and in the early stages of infection with either *Trypanosoma gambiense* or *Trypanosoma rhodesiense*, the examination of the cerebrospinal fluid is worthless. The trypanosomes are most numerous in the cerebrospinal fluid in those cases presenting grave nervous symptoms, as lethargy, and Reichnow (1921) has called attention to the fact that in this fluid many more dividing forms of the trypanosomes are found than in the peripheral blood.

The cerebrospinal fluid is secured by lumbar puncture and this may be performed with or without a general anæsthetic. Some authorities recommend that a general anæsthetic be used, but it is not necessary in the vast majority of cases. When properly performed, lumbar puncture is without danger provided the patient is not in a greatly debilitated condition. While many physicians do not insist upon the patient being in bed at the time of puncture, and remaining there for at least twenty-four hours afterward, I believe that this should always be insisted upon, and that lumbar puncture should never be performed unless the patient is in bed and kept there for twenty-four hours after the operation.

*Lumbar Puncture.* The lumbar region is carefully washed with soap and water, alcohol and ether, and the region over the lumbar vertebræ brushed with iodine solution. The site of the puncture is most easily ascertained by running the finger along the spines of the vertebræ until the so-called "soft spot" is reached, which is indicated by a distinct feeling of softness to the finger, and is situated between the third and fourth lumbar vertebræ. The patient may sit upon a stool with his back bent so as to bring the spinous processes of the vertebræ close to the surface, or lie upon the left side on the edge of the bed. The latter posture is necessary in weak or somnolent patients, but the sitting posture is suitable for ordinary ambulant patients. The needle used for puncture should be of flexible steel, measuring 10 cm. long, with a bore of 1 to 1.5 mm., while for a child the needle should be shorter, but of the same bore. The needle should be sterilized before use, as well as the stylet which should accompany it.

The space between the third and fourth lumbar vertebræ having been ascertained, the puncture is made directly in the median line, which I have found much more satisfactory than the lateral puncture recommended by some writers. The needle is held firmly and the puncture made quickly, the direction being straight forward. Enough force should be used to push the needle quickly through the skin and muscles, but as soon as the spinal ligaments are reached it should be pushed forward more slowly until there is a sudden sensation of loss of resistance when the needle is in the spinal canal. The stylet is now withdrawn

and the spinal fluid, if the puncture is successful, will flow from the needle, generally with some force. If the fluid does not flow, the needle may be gently shifted or the patient told to take a deep breath, or the stylet may be replaced in the needle and gently pushed forward to clear the bore of the needle, which may have become occluded by material forced into it by the influx of the spinal fluid. If none of these procedures are successful the puncture is called a "dry" one and there is little use in repeating it.

Not over 5 c.c. of fluid should be withdrawn unless it is ejected from the needle with much force, when as much as 10 c.c., or even more, may be obtained. The fluid should be collected in a clean glass graduate and centrifuged as soon as possible. The sediment obtained by centrifuging the fluid is examined in both fresh and stained preparations for the trypanosomes, as already described in the examination of the peripheral blood.

It should be remembered that this method of diagnosis is useless except in those infections in which well-marked nervous symptoms are present, and that the more marked these symptoms are the greater the number of trypanosomes that will be found in the cerebrospinal fluid.

**Inoculation of Susceptible Animals.**—Many animals are susceptible to infection with *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, and this fact may be taken advantage of in diagnosis where other methods have failed. Susceptible animals may be inoculated with the blood, cerebrospinal fluid, or gland juice of the suspected case and their blood examined at intervals to determine whether infection has occurred. This method of diagnosis has not been very widely employed owing to the fact that trypanosome infections can generally be diagnosed by the examination of the blood, gland juice, or cerebrospinal fluid, but it undoubtedly may prove useful where these other methods have failed, as such inoculations are often successful when the material inoculated is apparently free from trypanosomes, so far as a microscopical examination can demonstrate.

**Serum Diagnosis.**—Efforts have been made to diagnose infection with trypanosomes by means of various serum reactions, especially the agglutination and complement-fixation reactions, but, so far as infections with *Trypanosoma gambiense* and *Trypanosoma rhodesiense* are concerned, without any degree of success. A complement-fixation test has been evolved for the diagnosis of "dourine" in horses, caused by *Trypanosoma equiperdum*, which is of great practical value, but no such test has proven of service in the diagnosis of human trypanosomiasis.

The most useful methods employed in the diagnosis of trypanosome infections in man are the examination of the peripheral blood and of gland juice. In most instances of infection, especially in the early stages

of the infection, a careful microscopical examination of the blood will result in finding the parasites, especially if centrifuged specimens are examined, and where this fails, the examination of gland juice secured by puncture will generally result in success. The examination of the cerebrospinal fluid should always be reserved for cases showing nervous symptoms, and even in such cases only those presenting the most marked symptoms, as lethargy and coma, will give a high percentage of positive results. As the only hope of successful treatment of these infections lies in their early recognition and prompt treatment, it will be evident that the diagnosis of trypanosomiasis by examination of the cerebrospinal fluid is of little value, from a therapeutic standpoint, but the examination of the peripheral blood and of gland juice often results in the recognition of the infections at an early date, and when most amenable to treatment. For these reasons one should never neglect the examination of the blood and of gland juice, if enlarged glands are present, in any case of fever in a sleeping sickness locality or coming from such a locality.

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[Those who are interested in the literature of trypanosomiasis will find full abstracts and references to all the literature published, which is enormous in amount, in the *Sleeping Sickness Bulletin*, Vols. I-IV, 1908 to 1912, and the *Tropical Diseases Bulletin*, from Vol. I, 1912, to date.]

## CHAPTER IX

### THE BLOOD AND TISSUE FLAGELLATES OF MAN (CONTINUED).

#### SCHIZOTRYPANUM CRUZI. DOUBTFUL HUMAN SPECIES.

#### IMPORTANT TRYPANOSOMES OF LOWER ANIMALS.

In certain localities in South America a trypanosome occurs which causes a fatal disease in man and which is considered by the best authorities as belonging to a genus distinct from the genus *Trypanosoma*. The parasite is placed in a separate genus because of differences in morphology and life-history which appear to distinguish it from *Trypanosoma gambiense* and *Trypanosoma rhodesiense*.

#### Genus II. SCHIZOTRYPANUM, Chagas, 1909.

This genus was established in 1909 by Chagas to include a trypanosome which he discovered in a bug, *Triatoma megista*, and which he afterward found in man, and demonstrated to be the cause of an acute and chronic disease occurring in certain parts of Brazil.

#### Species I. SCHIZOTRYPANUM CRUZI, Chagas, 1909.

Synonyms: *Trypanosoma cruzi*, Chagas, 1909. *Trypanosoma escomeli*, Yorke, 1920. *Trypanosoma neotomæ*, Kofoed and McCulloch, 1916.

**History and Nomenclature.**—*Schizotrypanum cruzi* was discovered by Chagas in the intestine of a biting bug in Brazil, variously known as *Triatoma megista*, *Conorhinus megistus*, or *Lamprolaima megistus*. The infected bugs were allowed to bite a monkey and the same parasite was found in the blood of the bitten monkey. Continuing his observations, Chagas found the same trypanosome in the blood of a two-year-old child suffering from anemia accompanied by an irregular fever and enlargement of the lymphatic glands. Further observations by Chagas proved that the trypanosome, which he called *Schizotrypanum cruzi*, is the cause of a common infection in man occurring in Brazil and in some other countries of South America.

Owing to very distinct differences in its morphology and life-history, Chagas considered, and it is believed rightly, that this trypanosome is generically distinct from the other trypanosomes that have been found in the blood of man, and proposed for it the new generic name, *Schizotrypanum*. However, many authorities, as Fantham, Stephens, and Theobald (1916), and Brumpt (1922), continue to classify this parasite in the genus *Trypanosoma*. I believe that this organism should be placed in a distinct genus and that the generic name given it by Chagas should be adopted for trypanosomes answering in morphology and life-history to the type species, *Schizotrypanum cruzi*.

**Morphology.**—Unlike the species of trypanosomes already described, *Schizotrypanum cruzi* does not undergo multiplication in the peripheral blood of man by longitudinal division, but multiplies in the internal organs, especially in the striated muscles. The species is pleomorphic, the forms occurring in the peripheral blood differing markedly from those observed in the tissues of the internal organs and muscles.

In the peripheral blood of man the trypanosome is best studied in blood smears stained with the Wright or some other modification of the Romanowsky stain. The average length of the forms observed in the blood is about 20 microns, and two quite well-defined forms have been



FIG. 55.—*Schizotrypanum cruzi*. Trypanosome forms observed in the blood of experimentally infected cat. (After Hegner and Taliaferro.)  $\times 1,500$ .

noted occurring free in the peripheral blood, one, long and slender with a much elongated nucleus and kinetoplast, or blepharoplast; the other broad and short, with a round or oval nucleus and a kinetoplast or blepharoplast which is oval in shape and of large size. Both types have a broad undulating membrane and a free flagellum which extends considerably beyond the anterior end of the body. These forms have been considered by some observers as sexual in nature, but intermediate forms have been described by Brumpt (1922) and others, and there is no evidence of sufficient value to establish the sexual nature of the forms which have been described.

Many writers state that *Schizotrypanum cruzi* occurs both within the erythrocytes and free in the blood plasma, but I have not been able to confirm the intracellular situation of the trypanosome in the material that I have examined. I have observed many instances in which the parasite was apparently contained within a red blood corpuscle, but careful examination has always convinced me that in every such instance the parasite was superimposed upon or attached to the cell rather than developing within it. Often the parasite appears to be coiled up in the erythrocyte, but this is due, I believe, to pressure in making the blood smears which has resulted in distorting the trypanosome and attaching it to the red corpuscle. Many such coiled trypanosomes are observed free in blood smears, and it is frequently observed that forms showing displacement of the nucleus and blepharoplast, as well as par-

tial separation of the undulating membrane, are common in blood smears, indicating that this trypanosome is easily distorted during the preparation and staining of the smears.

The forms of *Schizotrypanum cruzi* occurring in the cells of the internal organs and in the striated muscles differ greatly in morphology from those seen in the peripheral blood. In the tissues the forms observed resemble *Leishmania*, being small oval or round bodies, measuring from 1.5 to 5 microns in diameter, and containing two definite chromatin staining masses, one large and oval in shape, the nucleus, and one very minute and rod-like or dot-like in appearance, the blepharoplast or kinetonucleus. These bodies occur in clumps in the cells of the internal organs and striated muscles and vary somewhat in size, the larger forms being most numerous, but in individual clumps the parasites are generally of the same size. It is believed that the larger forms are the multiplying forms in the tissues, while the small forms are those from which the trypanosome form, as observed in the peripheral blood, develop.

In experimental animals the development of the trypanosome form seen in the peripheral blood from the forms found in the tissues can be traced, the leishmania-like forms developing an undulating membrane and flagellum, and in such animals one may find leishmania, herpetomonad, crithidial, and trypanosome forms of the parasite in the tissues.

In rare instances, leishmania-like forms may be observed in the peripheral blood in experimental animals, but such forms do not occur in the blood of man. Multiplication of the trypanosome only occurs in the cells of the internal organs or the striated muscles, and when such forms occur in the peripheral blood it must be regarded as due to an accidental invasion of the blood stream by the undeveloped parasites.

In fresh specimens of blood, *Schizotrypanum cruzi* is actively motile, moving about in an irregular manner and quite unlike other mammalian trypanosomes, in that the motility is not so rapid, and a definite direction is seldom maintained for any distance. The trypanosome is colorless and details of structure cannot be seen except in those organisms in which motility has almost ceased, when the undulating membrane and flagellum may be distinguished.

**Habitat.**—This parasite lives in the peripheral blood, tissues, and muscles of man, in the intestine of certain insects, and, according to Chagas, Brumpt, and others, in certain species of armadillos, which they believe act as reservoirs of the virus. In the peripheral blood of man *Schizotrypanum cruzi* can be demonstrated for only fifteen to thirty days during the acute symptoms, and is found in the tissues of the internal organs and in the striated muscles in the chronic infections.



The parasite has also been found in the blood of the domestic cat in infested localities.

No other species of *Schizotrypanum* has been described as occurring in any of the lower animals.

**Cultivation.**—*Schizotrypanum cruzi* can be artificially cultivated, and the forms observed in cultures are similar to those observed in the transmitting insect, *Triatoma megista*.

The culture medium which has proven most successful in the cultivation of this parasite is the N.N.N. medium used in the cultivation

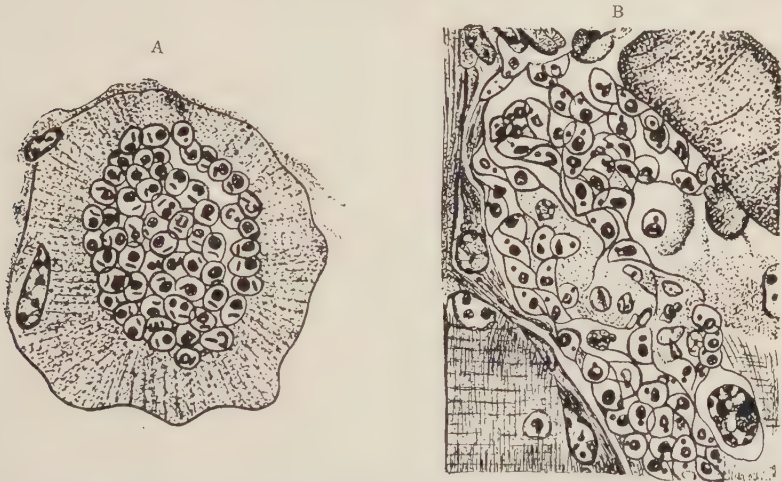


FIG. 56.—*Schizotrypanum cruzi*. A. Transverse section of striped muscle fibre invaded by *Schizotrypanum cruzi*. (After Vianna.) B. Section of invaded muscle in which *S. cruzi* has caused degeneration of the fibres. Some of the trypanosomes have a flagellum. (After Vianna.)

of *Leishmania*. The parasite may be kept alive for long periods on this medium and cultivated through many generations.

Nöller (1920) was able to keep *Schizotrypanum cruzi* alive on plate cultures for over one year and obtained the best results if the cultures were kept at a temperature of 30° C., although he obtained excellent results at room temperature. In cultures kept at 37° C. the trypanosomes degenerated. In Nöller's cultures, most of the forms observed resembled *Leishmania*, but true trypanosome forms were sometimes encountered. The cultures were virulent for mice at the end of twelve months.

Recently Torres (1922) has obtained cultures of *Schizotrypanum cruzi* in meat broth containing 7 per cent. of sodium chloride and 5 per cent. of peptone and having a reaction between pH 6.55 and pH 7.18. Abundant growth was obtained in this medium and the trypanosomes remained alive for from twenty-four to forty-nine days. Torres found that oxygen was essential for the growth of the trypanosomes, as no growth occurred in the medium if the tubes containing it were sealed with

liquid vaseline, while in medium in unsealed tubes, growth was abundant in from twenty to thirty days, and the cultures were virulent for guinea-pigs after forty-nine days.

**Life-history.**—The life-history of *Schizotrypanum cruzi* includes two cycles of development, one within man and one within the transmitting insect. There is, as yet, no general agreement regarding the exact cycle of development in either host, and the description which follows is based upon the observations of Chagas, Hartmann, Brumpt, and others.

As already stated, no multiplication of the trypanosome forms occurs in the peripheral blood, but these forms are believed to lose their flagellum and undulating membrane and to enter the tissue cells, where they multiply, according to Hartmann (1910), by schizogony. He describes intracellular forms in the lungs of experimentally infected guinea-pigs containing many nuclei and blepharoplasts which eventually divide into numerous daughter parasites which become true trypanosome forms and appear in the peripheral blood. Similar forms have been described as occurring in the tissues of man, and it has been noted that there is a more or less definite increase in the number of parasites in the peripheral blood at certain intervals which may correspond to the schizogony of the parasites in the internal organs.

Chagas has worked out the cycle of development of *Schizotrypanum cruzi* in *Triatoma megista*, the most common transmitting insect, and for this purpose found that the young larvæ gave the best results, as the fully developed insect was almost invariably infected with other intestinal flagellates which confused the picture and made the results inaccurate. He found that about six hours after the larva has fed upon blood containing the trypanosome the parasites lost their undulating membrane and flagellum, became round in shape, and increased in number. At first situated in the anterior portion of the mid-gut, the parasites quickly leave this portion of the gut for the cylindrical portion, where multiplication occurs rapidly and a crithidial stage is developed which persists in this portion of the gut for long periods of time. Chagas has found developmental stages in the salivary glands of *Triatoma megista*, but such invasion occurs only in about one per cent. of the bugs which have been examined from infested houses. Chagas believes that the invasion of the salivary glands occurs through the body cavity and has found the parasite in the body cavity of bugs caught in infested houses, but very rarely.

The duration of the life-cycle, either in man or the transmitting insect, is unknown, but larvæ of *Triatoma megista* become infective in from eight to ten days after feeding upon blood containing the trypanosome.

**Geographical Distribution.**—So far as is known at present, the geographical distribution of *Schizotrypanum cruzi* is confined to South America, and to only three countries in that continent, *i.e.*, Brazil, Venezuela, and Peru. Chagas first discovered the trypanosome in children in the state of Minas Geraes, in Brazil, but it has been found also in Bahia, Goyaz, Piauh, and San Paulo, Brazil, and in 5 Miranda, Trujillo, and Zulia, Venezuela. Escomel has described a trypanosome occurring in Peru which is apparently identical with *Schizotrypanum cruzi*, but which Yorke (1920) accepts as a new species for which he has proposed the name, *Trypanosoma escomeli*.

Although this trypanosome has been demonstrated in man in only the localities mentioned, it has been found that bugs belonging to the genus *Triatoma* and naturally infected with a trypanosome which, upon injection into susceptible animals, gives rise to the same lesions as *Schizotrypanum cruzi*, occur not only in the localities mentioned above, but also in San Salvador, the Argentine Republic, and Colombia, and it is probable that human infections with the parasite will eventually be discovered in these localities. Bugs belonging to the genus *Triatoma* and to other genera which contain species which have been found capable of harboring this trypanosome are numerous throughout tropical America, and are potential carriers of the infection, so that a far wider distribution of the parasite may be expected in the future.

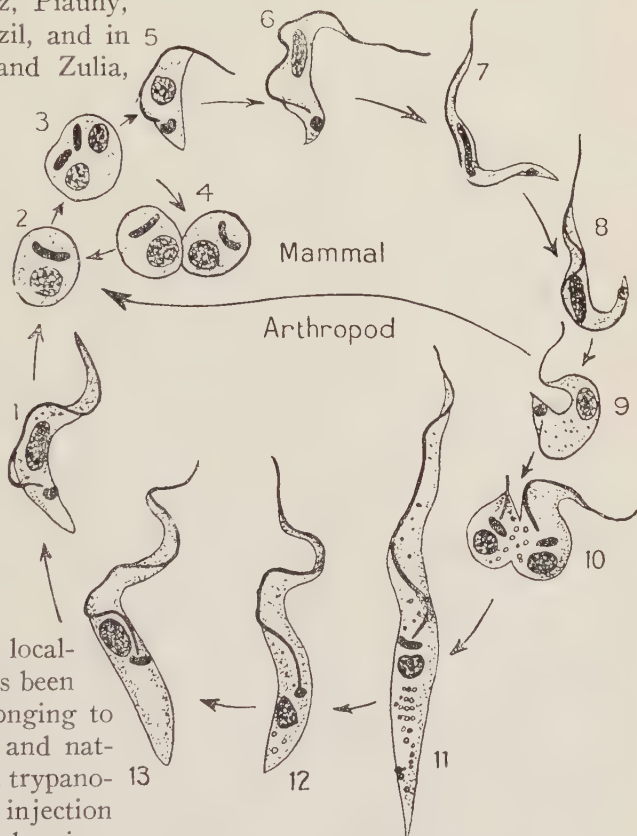


FIG. 57.—Life-cycle of *Schizotrypanum cruzi*. (After Brumpt.) 1. *Schizotrypanum* from posterior intestine of *Triatoma* inoculable into mammals. 2 to 6. Leishmania and trypanosome forms developing in the tissues of mammals. 7. Young, slender, mobile form. 8. Intermediate form. 9. Adult form capable of being inoculated into susceptible animals or of infecting certain insects. 10. Crithidial dividing form. 11. Large crithidial form. 12. Transitional form, quite similar to typical trypanosome. 13. Young trypanosome form.

**Incidence of Infection.**—According to Chagas, infection with *Schizotrypanum cruzi* is wide-spread in certain localities, practically the entire population being infected. Sporadic infections have been observed in other localities where apparently conditions are not favorable to the transmission of the parasite.

**Method of Transmission.**—Chagas (1909) discovered *Schizotrypanum cruzi* in the intestine of *Triatoma megista*, a reduvid bug which lives in the walls of the huts of the poorer classes in Brazil, later demonstrating that this trypanosome is pathogenic to man, and that the most common transmitting agent is *Triatoma megista*. The bug is called “barbeiro” by the natives, and bites at night, children being very frequently bitten. It lives from one to two years and remains infective during this entire period. The larvæ, nymphs, and adults may convey the infection experimentally, but there is no evidence that the infection is hereditary in the bugs. In nature the infection of man is usually by the adult insect. After biting an infected individual the bug becomes infective in from eight to ten days. The life-cycle of the trypanosome in the bug has already been considered.

Although *Triatoma megista* is the common transmitting insect, other species of *Triatoma* have been found naturally infested with *Schizotrypanum cruzi*. Thus *Triatoma geniculata*, *T. sordida*, *T. dimidiata*, *T. vitticeps*, *T. chagasi*, and *T. braziliensis* have all been found naturally infested and are potential transmitters of the trypanosome and, undoubtedly, act as such in the regions in which the individual species occur.

In Venezuela, as proven by Tejera (1919), the transmitting insect is *Rhodnius prolixus*. This bug is found to be naturally infested, and inoculations of infective material from this bug into susceptible animals was found by Tejera to be followed by the appearance in the inoculated animals of a trypanosome indistinguishable from *Schizotrypanum cruzi*. He also demonstrated that the larvæ of *Rhodnius prolixus*, when fed upon animals infected with this trypanosome, developed flagellated forms in their intestinal tract identical with those found in the naturally infested insect. *Triatoma megista* does not occur in Venezuela.

In the United States a species of *Triatoma*, *T. sanguisuga*, occurs in Texas and California, where it is known as the “kissing bug” because of the fact that it frequently bites the lips of its victims. Brumpt (1922) has shown that this species is readily infected experimentally with *Schizotrypanum cruzi* and it is, therefore, a potential transmitter of the infection. He has also shown that the cosmopolitan species, *Triatoma rubrofasciata*, can be easily infected experimentally. Thus, under favorable conditions, infection with this trypanosome may become prevalent in the United States.



The interesting discovery by Kofoed and McCulloch of a trypanosome, *T. neotomæ*, in *Triatoma protracta*, a bug occurring in California, is important in this connection, as Brumpt (1922) considers that *T. neotomæ* may be identical with *Schizotrypanum cruzi*.

Though bugs belonging to the genus *Triatoma* are generally the transmitting agents of *Schizotrypanum cruzi*, other bugs may be experimentally infected. The bed-bugs *Cimex lectularius*, *C. boueti*, *C. rotundatus*, *C. hemiptera*, and *C. hirundinus*, and the ticks *Ornithodoros moubata* and *Rhipicephalus sanguineus* have been infected experimentally and the trypanosome has been shown to develop in the posterior part of the intestine of these insects. Furthermore, Neiva and Pinto (1923) have proven that *Rhipicephalus sanguineus* and *Amblyomma cayennense* are able to transmit the infection mechanically.

It is very difficult to transmit the infection by the bite of either bed-bugs or ticks, but the fæces of these insects are infective.

There is much difference of opinion as to the exact manner in which the insect transmits the trypanosome, but it is probable that infection may occur directly through the bite of an infested insect or indirectly through the dejecta. Brumpt (1919) states that infection generally occurs through the dejecta of the insect being rubbed or scratched into the lesion produced by the bite of the insect, and only rarely by means of trypanosomes contained in the secretion from the salivary glands. It has been conclusively demonstrated that the intestinal contents and fæces of infested bugs are very rich in trypanosomes and that, when biting, the bugs defecate, so that infection of the wound caused by biting can easily occur, and it is probable that this is the most frequent method of transmission. However, the observations of Chagas, which demonstrated that the trypanosomes may be found in the salivary gland, indicate that infection may occur through the injection of material from these glands. Torres (1913) was successful in infecting kittens by allowing *Triatoma megista* to bite them, the experiments being conducted in such a way that it was impossible for the fæces of the insects to come in contact with the kittens, and obtained nineteen positive results in thirty-five animals experimented upon.

It has been found that only a small proportion of the experiments in infecting *Triatoma megista* by allowing the insects to bite individuals or animals infected with *Schizotrypanum cruzi* are successful, little over one per cent. of the insects becoming infective.

**Transmission by Milk.**—Nattan-Larrier (1913) first demonstrated that *Schizotrypanum cruzi* constantly passes into the milk of infected animals in an infective condition, and his observations have been confirmed by others. Whether the ingestion of such milk would be followed by infection in man is undecided, but if the milk of an infected mother

contains the trypanosomes it is possible that infection might occur by the parasites reaching open lesions on the lips or in the mouth of the nursing child, but such a method of transmission has not been proven and is very problematical.

**Transmission by Coitus.**—During experiments with guinea-pigs, Vianna found that *Schizotrypanum cruzi* could sometimes be demonstrated in the semen of infected animals, and Nattan-Larrier (1921) has shown that infection by coitus is possible. He introduced into the healthy vagina of mice material containing this trypanosome and secured infection in the three animals experimented with in this manner. No abrasions were necessary in order to produce infection, the trypanosomes penetrating the uninjured vaginal mucous membrane. He also found that infection could occur through the conjunctivæ and the intestinal mucous membrane.

**Hereditary Transmission.**—Nattan-Larrier (1921) has shown by experiments with guinea-pigs that *Schizotrypanum cruzi* can be transmitted from the mother to the foetus through the amniotic fluid, but hereditary transmission of the infection in man has not been demonstrated nor does it occur clinically.

**Reservoirs of the Virus.**—Chagas, Brumpt, and others believe that armadillos serve as the natural reservoirs of *Schizotrypanum cruzi*, and that *Triatoma geniculata*, a bug living in the burrows of the armadillo, acts as the transmitting agent. Over 50 per cent. of armadillos examined have shown infection with *Schizotrypanum cruzi*, the trypanosome being found in the peripheral blood and internal organs. Several species of armadillo have been found naturally infected, i.e., *Tatus novemcinctus*, *Dasypus sexcinctus*, and *Dasypus unicinctus*. Crowell (1923) has recently described *in extenso* the occurrence, in a naturally infected armadillo, of *Schizotrypanum cruzi* in the blood, the myocardium, and the cardiac muscle fibres.

From the evidence that has accumulated there would appear to be little doubt that the armadillo acts as the natural reservoir of *Schizotrypanum cruzi*.

**Experimental Infection of Lower Animals.**—Most of the small laboratory animals are susceptible to experimental infection with *Schizotrypanum cruzi*. Guinea-pigs, rats, mice, lemurs, cats, many species of monkeys, and the chimpanzee, can all be easily infected experimentally, and cats are found naturally infected. Singing birds and domestic fowls are immune according to Brumpt and others. It is stated that, while experimentally infected monkeys are infective to the bug, *Triatoma megista*, it is impossible to infect the bug from experimentally infected guinea-pigs.

It was from the study of the trypanosome in experimentally infected laboratory animals that the life-history has been gradually worked out by Chagas, Brumpt, Mayer and da Rocha-Lima, and others, and what is known regarding the method of transmission we also owe to animal experimentation.

**Relation to Disease.**—*Schizotrypanum cruzi* is the cause of an acute and chronic infection especially prevalent among children in the localities in which this trypanosome has been found to occur. Infection with this parasite is unique as regards the history of the discovery of the parasite, as Chagas first discovered it in the insect transmitting the infection rather than in the host in which it produces disease.

Chagas describes an acute and chronic type of infection with *Schizotrypanum cruzi*, the acute form occurring in young children usually during the first year of life, the chronic form following in children that survive, or the disease may be chronic in the adult from the beginning. The acute form is characterized by fever, myxedema, enlargement of the lymph glands, the spleen, liver, and thyroid gland. Keratitis is a common and characteristic symptom. The trypanosomes are found in the peripheral blood during the acute stage of the disease and are sometimes very numerous.

The acute stage is very often fatal, lasting from three to four weeks. If the child survives, the chronic stage of the infection supervenes, in which there are febrile attacks and symptoms referable to localization of the trypanosomes in the viscera are noted. Thus Chagas describes myxedematous, cardiac, suprarenal, and nervous types of the disease, and acute exacerbation occurs in all of these types, during which time fever is present.

In the chronic stage of the infection the trypanosomes are not found microscopically in the peripheral blood, although injections of blood into susceptible animals are sometimes followed by infection. The trypanosomes occur in the internal organs during the chronic stage, especially in the cardiac muscle fibres.

The pathology of the infection has been very thoroughly studied by Chagas and Vianna (1911) and Mayer and da Rocha-Lima (1912), and it has been found that almost every tissue in the body may be invaded by the parasite. Probably the most widely invaded of the tissues are the cardiac and skeletal muscles, but the parasites may also be found in the spleen, liver, suprarenals, lymph glands, adenoid tissue, fatty tissues, the submucosa of the mucous membranes, the ovaries, uterus, testis, and in the neuroglia cells of the central nervous system. The bone marrow, connective tissue, and epidermis are less commonly invaded.

There is apparently no natural immunity to the infection and acquired immunity is doubtful.

**Diagnosis.**—The diagnosis of the infection must rest upon the finding of *Schizotrypanum cruzi* in the peripheral blood or in the tissues. The methods for the examination of the peripheral blood for *Trypanosoma gambiense* and *Trypanosoma rhodesiense* already described are applicable for the diagnosis of this infection. If the peripheral blood is negative microscopically, as it is in all but the acute cases, inoculation of the blood into guinea-pigs should be employed as a diagnostic measure, and this method is often successful in the chronic stage of the infection. If nervous symptoms are present the inoculation of the cerebrospinal fluid into guinea-pigs is sometimes successful in producing an infection.

Brumpt has urged the value of the employment of the transmitting insect, *Triatoma megista*, or other species of *Triatoma* in the diagnosis of the infection. If the bug is allowed to bite an individual suffering from the infection it is almost invariably infected and an examination of the bug will result in the detection of the parasite. This method of diagnosis he calls "Xenodiagnosis." It is obvious that one must be sure that the bug is not already naturally infected when allowed to bite, and for this reason only bugs reared in captivity should be used. Infection of the bug occurs even though the trypanosomes are so few in number in the blood that they cannot be demonstrated microscopically. The method would appear to be a valuable one in the hands of experts, but it is doubtful if it could be employed except under exceptional circumstances.

**Prophylaxis.**—The prophylaxis of this infection consists of the destruction of the transmitting insects and protection from their bites. As the transmitting insects live in the walls of the habitations of the poorer classes, because of the poor construction and material of their houses, it is obvious that a most important prophylactic measure would be the construction of better habitations. Fumigation should be employed if possible in order to destroy the insects but, owing to the nature of the infested habitations, little short of destruction is efficient in ridding the houses of the bugs.

The use of mosquito nets is an efficient method of prophylaxis, and such nets should be used in localities where *Triatoma megista* is present.

### DOUBTFUL TRYPANOSOMES OF MAN

There have been several species of trypanosomes reported as causing disease in man which must be regarded as doubtful. Most of them are undoubtedly identical with one or the other of the trypanosomes that have already been described and have been included in the synonyms



under the descriptions of the respective organisms. The following may be mentioned:

1. TRYPANOSOMA CASTELLANII, Kruse, 1903.

This trypanosome was first observed by Castellani in the cerebro-spinal fluid of cases of sleeping sickness and was described by Kruse, in 1903, as the cause of sleeping sickness in Uganda and named by him. It is undoubtedly identical with *Trypanosoma gambiense*.

2. TRYPANOSOMA VIVAX, var. MACFIENSIS,  
Castellani and Chalmers, 1920.

In 1917, Macfie found a trypanosome in the blood of a man suffering from trypanosomiasis on the Gold Coast which agreed in its morphology with *Trypanosoma vivax* except that it was smaller, measuring 21 microns in length instead of 23 microns. The maximum length is given as 24 microns, the minimum as 18 microns. It is monomorphic, the body narrowing abruptly just anterior to the nucleus (triphonucleus). The posterior extremity is blunt, containing a large terminal blepharoplast. It has a delicate narrow undulating membrane and a long free flagellum. It has been found in only one case in man. Its exact status is still undetermined, but it is probably identical with *Trypanosoma vivax*.

3. TRYPANOSOMA NIGERIENSE, Macfie, 1913.

This trypanosome was described by Macfie, in 1913, who found it in children and young adults in the Eket region of Southern Nigeria. It is stated to cause a mild type of trypanosomiasis in this region, which does not occur in epidemics.

Morphologically it is practically identical with *Trypanosoma gambiense*, although some forms are described as being shorter than *gambiense* and, according to Macfie, a flagellum free throughout its entire length is sometimes observed. In experimental animals, very short forms were observed, measuring less than 8 microns in length, and in some forms the nucleus (triphonucleus) was situated more anteriorly than in *gambiense*.

Recent authorities are united in considering that *Trypanosoma nigeriense* is identical with *Trypanosoma gambiense*.

4. TRYPANOSOMA ESCOMELI, Yorke, 1920.

This trypanosome was found in the blood of a man suffering repeated attacks of fever accompanied by anæmia and œdema, by Escomel (1919), in Peru. He considered it to be identical with *Schizotrypanum cruzi*, but Yorke considers that it is a distinct species and named it

*Trypanosoma escomeli*. According to Yorke, it is much longer than *Schizotrypanum cruzi*, while the blepharoplast is much more minute. The exact specific status of this parasite is still undecided.

5. TRYPANOSOMA CRUZI, var. SEGOVIA, Segovia, 1914.

A trypanosome found in the blood of a patient suffering from irregular fever and erythema which morphologically resembled *Schizotrypanum cruzi*. Segovia, who described the parasite, considered that it differed from *cruzi* in some particulars, and proposed the name *Schizotrypanum cruzi*, var. *Segovia*, for it. It is probably identical with *Schizotrypanum cruzi*.

IMPORTANT TRYPANOSOMES OF LOWER ANIMALS

Owing to the fact that practitioners of medicine in the tropics are frequently brought into contact with animals suffering from trypanosomiasis through consultation, in the absence of veterinary surgeons, it is important that they be able to diagnose the most important of the trypanosomes causing serious disease in stock, and for this reason the following brief descriptions of the more important trypanosomes of some of the lower animals are included in this chapter. A list of these trypanosomes, with their synonyms, will be found on page 233.

1. TRYPANOSOMA BRUCEI, Plimmer and Bradford, 1899.

This trypanosome was discovered in 1894, by Sir David Bruce, who found it in cattle in Zululand suffering from a fatal disease known as "nagana." The disease also occurs in horses, mules, donkeys, dogs, and cats, and is widely distributed in Africa. The infection is transmitted from animal to animal by *Glossina morsitans* and other species of *Glossina*. The reservoirs of the infection are big game animals, as bushbuck, wildebeest, and koodoo, and in these animals the trypanosome is apparently harmless.

**Morphology.**—*Trypanosoma brucei* varies in length from 12 to 35 microns and in breadth from 1.5 to 4 microns. It multiplies by longitudinal division in the peripheral blood. In morphology it is practically identical with *Trypanosoma rhodesiense*, being very pleomorphic and showing short, broad forms having a posterior nucleus.

**Pathogenicity.**—*Trypanosoma brucei* is the cause of a very fatal disease in cattle and horses known as "nagana," characterized by anæmia, emaciation, fever, and œdema of the subcutaneous tissues of the neck, abdomen, and limbs. The experimental infection of the small laboratory animals is invariably followed by death and the trypanosomes occur in their blood in enormous numbers. Novy and MacNeal were success-

ful in cultivating this trypanosome upon blood agar, and their work has been confirmed by numerous other investigators.

## 2. *TRYPANOSOMA CONGOLENSE*, Broden, 1904.

This trypanosome was first found in domestic stock in the Congo by Broden, in 1904, but is now known to be generally distributed throughout Central Africa, where it causes the disease in horses known as "Gambian horse sickness."

**Morphology.**—It is a monomorphic trypanosome measuring from 7 to 14 microns in length and from 1.5 to 2 microns in breadth. The nucleus is placed near the centre of the body and is round or oval in shape, the blepharoplast, or kinetonucleus, being situated near the posterior extremity of the body, and of a spherical shape. The undulating membrane is very narrow, the flagellum forming the outer border being close to the body and not thrown into well-marked folds. A free flagellum is never observed.

**Pathogenicity.**—In nature, both equidæ and ruminants are found infected with *Trypanosoma congolense*, and in native animals it is not of great pathogenic importance. The inoculation of the trypanosome into European horses, cattle, or sheep is generally followed by fatal results, but in small laboratory animals infection only occurs in a small proportion of inoculation experiments. However, when a strain has become established in one of the small laboratory animals it is possible to carry it along for many generations, and passage from animal to animal greatly increases its virulence, as shown by Blacklock and Yorke.

This trypanosome has not been artificially cultivated. It is transmitted by *Glossina morsitans*.

## 3. *TRYPANOSOMA EQUINUM*, Voges, 1901.

Elmassian discovered this trypanosome in the blood of horses suffering from a disease known as "mal de caderas" in South America. Infection with *Trypanosoma equinum* occurs in Brazil, Argentine Republic, Bolivia, Paraguay, and in other localities in South America.

**Morphology.**—*Trypanosoma equinum* is a monomorphic trypanosome measuring from 25 to 55 microns in length and from 1.5 to nearly 2 microns in breadth. The nucleus is situated centrally, while the blepharoplast is generally invisible, owing to its minute size. When visible the blepharoplast is situated at the posterior extremity of the body. There is a broad, much curved undulating membrane, and a free flagellum of medium length. The blepharoplast is said to be absent, by many observers, but in the strains that I have examined, while many trypanosomes were observed without a blepharoplast, many possessed a minute gran-

ule which I believe is a blepharoplast, although it might be interpreted as the basal granule, in some instances, owing to its very minute size.

**Pathogenicity.**—*Trypanosoma equinum* is the cause of a fatal disease in horses, but may be experimentally inoculated into all domesticated animals with fatal results. The small laboratory animals are easily infected experimentally, and the trypanosome causes a rapidly fatal disease in them.

The method of transmission of this trypanosome is still unknown. The biting flies, *Stomoxys nebulosa* and *Stomoxys calcitrans*, as well as various species of *Tabanida*, have been regarded as the transmitting insects by various observers, while Neiva (1913) considers that *Crysops* or *Triatoma* may transmit the parasite.

The reservoir of infection is believed by Migone and others to be a rodent, the capybara (*Hydrochærus capybara*), as this animal is found naturally infested with *Trypanosoma equinum*.

#### 4. TRYPANOSOMA EQUIPERDUM, Doflein, 1901.

This important trypanosome was discovered by Rouget, in 1896, in the blood of a horse, in Algeria, suffering from a disease known as "dourine" or "mal du coit." It is widely distributed, having been found in horses in many countries of Europe, in North Africa, India, and North America. Numerous epidemics have occurred in horses in the United States. The trypanosome was named by Doflein, in 1901.

**Morphology.**—*Trypanosoma equiperdum* is a monomorphic trypanosome measuring from 24 to 28 microns in length, on the average, but forms are observed as short as 15 microns and as long as 36 microns. The nucleus is situated centrally and is oval in shape, while the blepharoplast is situated at the posterior extremity of the body and is spherical in shape. Metachromatic granules, so frequently observed in stained preparations of other trypanosomes, are generally entirely absent in this species. The undulating membrane is well marked, being very broad and folded, and there is a well-defined flagellum of considerable length.

**Pathogenicity.**—*Trypanosoma equiperdum* is confined, in nature, to the horse and ass, producing in these animals a fatal disease which occurs in an acute and chronic form, the acute form terminating fatally in from one to two months, while the chronic form may last for several months or a year. The trypanosomes occur in small numbers in the blood but are common in the œdematous plaques or tissues about the genital organs, which are characteristic of the infection.

The infection is transmitted entirely by coitus and has been termed syphilis of horses by some investigators. The small laboratory animals can be experimentally infected with some difficulty. Complement-fixing bodies are produced in the blood of infected horses and the complement-



fixation test is now a well-recognized and widely employed method of diagnosis in this disease.

### 5. *TRYPANOSOMA EVANSI*, Steel, 1885.

*Trypanosoma evansi* was discovered by Evans in the blood of horses in India suffering from a disease known as "surra." The same trypanosome has also been found in cattle, camels, and dogs. It has a wide geographical distribution, having been found in domestic animals in India, Burma, Indo-China, Java, the Philippines, Mauritius, Federated Malay States, Netherland Indies, and in imported animals in Australia and the United States. In the Philippines it was the cause of serious loss of horses during military operations connected with the Philippine Insurrection.

**Morphology.**—*Trypanosoma evansi* is a monomorphic trypanosome resembling very closely in morphology *Trypanosoma gambiense*. In length it varies from 18 to 34 microns and in breadth from 1.5 to 2 microns. The posterior extremity is pointed, while the anterior extremity possesses a long, free flagellum. The nucleus is central in position, and is oval in shape. The blepharoplast, or kinetonucleus, is spherical in shape and situated near the posterior extremity. The undulating membrane is folded and broad, and terminates in the free flagellum. Leishmania-like forms are observed in the spleen of infected vertebrates which Walker (1912) considers are stages in the life-cycle, and the result of schizogony. In the blood, shorter forms are rarely observed similar to those of *Trypanosoma gambiense*. The long, slender forms and the short, broad forms have been considered by some observers to be sexual in nature.

**Pathogenicity.**—This trypanosome is the cause of fatal disease in all domestic animals, but most commonly produces a disease in horses known as surra, characterized by fever, anæmia, œdematous swellings, paralysis, and a fatal ending. A similar disease occurs in dromedaries in Africa, which is caused by a closely related trypanosome called *Trypanosoma evansi*, var. *mboi*, while in the Sudan and Algeria a disease called "el debab" and "tahaga" in camels is caused by another strain, or variety, of this trypanosome called *Trypanosoma soudanense*.

The exact method of transmission of *Trypanosoma evansi* is still unknown, although many insects have been suspected. In the Philippines a careful study of the transmission of the infection led me to believe that *Stomoxys calcitrans*, the common stable fly, was the transmitting agent. There is little doubt that the trypanosome is transmitted mechanically by various species of *Tabanus* and *Stomoxys*, and Mitzmain, in the Philippines, has obtained successful infections in animals through the bite of *Tabanus striatus*. There is no evidence that the trypanosome

passes through a cycle of development in these flies, but the evidence is conclusive that infection may occur directly by their bites provided not too long an interval is allowed between an infected feed and the biting of the uninfected animal.

The small laboratory animals are easily infected with this trypanosome and the infection is fatal in all within a short period of time.

#### 6. *TRYPANOSOMA HIPPICUM*, Darling, 1910.

This trypanosome was discovered by Darling in the blood of mules imported into Panama from the United States and suffering from a disease known as "murrina."

**Morphology.**—*Trypanosoma hippicum* is a monomorphic trypanosome resembling in morphology *Trypanosoma evansi*. It measures from 18 to 28 microns in length and from 1.5 to 3 microns in breadth. The nucleus is round or oval, and situated centrally, while the blepharoplast is round, and situated at the posterior end of the organism. The undulating membrane is less folded than that of *Trypanosoma evansi*, and there is a free flagellum of considerable length.

**Pathogenicity.**—It produces the disease in mules known as "murrina" and is easily inoculable into the small laboratory animals, producing in them an acutely fatal infection. Laveran has proven by cross immunity experiments that this trypanosome is distinct from *Trypanosoma evansi*.

The method of transmission is still in question, but it is believed that it is transmitted by coitus, and by the bites of certain species of flies belonging to the genera *Musca*, *Comptosmyia*, and *Sarcophaga*.

#### 7. *TRYPANOSOMA VIVAX*, Ziemann, 1905

*Trypanosoma vivax* was discovered by Ziemann in the blood of cattle, sheep, and goats suffering from "souma" in the Cameroons. It is a widely distributed species in all parts of tropical Africa.

**Morphology.**—This trypanosome is monomorphic and measures from 16 to 31 microns in length by 2 to 3 microns in breadth. The nucleus is in the centre of the body, and is oval in shape, while the blepharoplast is round, and situated near the posterior extremity. The undulating membrane is narrow and little folded so that the flagellum forming the outer edge of this membrane is rather closely applied to the body. There is a free flagellum of considerable length. Forms are observed which are broader than the average and which have a short, free flagellum.

**Pathogenicity.**—*Trypanosoma vivax* is the cause of a disease in cattle, sheep, and goats known as "souma" and which in native animals is of slight virulence, but if the trypanosome be transmitted to European animals the disease is usually fatal. Small laboratory animals are resistant to infection, but rats and rabbits have been infected.

This trypanosome has been found once in the blood of a healthy native of the Gold Coast by Macfie (1917).

The trypanosome is transmitted by species of *Glossina*, and *Glossina palpalis*, *Glossina morsitans*, and *Glossina tachinoides*, have all been proven experimentally to be capable of transmitting *Trypanosoma vivax*.

**Other Species of Trypanosomes.**—Numerous other species of trypanosomes have been described in mammals, but are of relatively little importance. Among these may be mentioned *Trypanosoma lewisi*, Kent, 1879, of the rat; *Trypanosoma guyanense*, Leger and Vienne, 1919, found in cattle in French Guiana and Venezuela; *Trypanosoma theileri*, Laveran, 1902, found in the blood of cattle throughout the world; *Trypanosoma capræ*, Kleine, 1910, of goats in Africa; and *Trypanosoma nanum*, Laveran, 1905, a species causing disease in cattle along the White Nile.

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## CHAPTER X

### THE BLOOD AND TISSUE FLAGELLATES OF MAN (CONTINUED). THE LEISHMANIA. *LEISHMANIA DONOVANI*. DIAGNOSIS OF INFECTIONS WITH *LEISHMANIA DONOVANI*.

The *Leishmania* are important pathogenic flagellates of man belonging to the PROTOZOA, family TRYPANOSOMIDÆ, and subfamily HERPETOMONINÆ. In the subfamily HERPETOMONINÆ four distinct genera may be recognized, *i.e.*, *Herpetomonas*, *Leptomonas*, *Crithidia*, and *Leishmania*. Of these genera the only one containing flagellates that are parasitic in man is the genus *Leishmania*.

#### Genus LEISHMANIA, R. Ross, 1903.

Synonyms: *Piroplasma*, Laveran and Mesnil, 1903. *Helcosoma*, Wright, 1903. *Herpetomonas*, Rogers, 1904.

The genus *Leishmania* was founded by Ross, in 1903, to include the causal organism of kala-azar, discovered by Leishman, in 1900. As it is certain that this organism is really a herpetomonad the validity of the genus *Leishmania* has been called in question by many authorities, and at the present time is still a matter of controversy. In the opinion of many observers, all the flagellates now included in the genus *Leishmania* should be transferred to the genus *Herpetomonas* and the genus *Leishmania* abolished, but it would appear to be best at present to retain this genus, as it has become firmly fixed in the literature, but with the understanding that the flagellates included in it are most probably herpetomonads that have become parasitic in man.

The genus *Leishmania* contains two generally accepted species that are parasitic in man, *Leishmania donovani* and *Leishmania tropica*, and one species, *Leishmania infantum*, which is now believed to be identical with *Leishmania donovani*. In addition there are one or more doubtful species or varieties.

#### Species I. LEISHMANIA DONOVANI, Laveran and Mesnil, 1903.

Synonyms: *Piroplasma donovani*, Laveran and Mesnil, 1903. *Herpetomonas donovani*, Ross, 1904. *Leishmania infantum*, Nicolle, 1908.

**History and Nomenclature.**—This flagellate was discovered in 1900, by Sir William Leishman, in smears from the spleen of a soldier who died at Netley from a fever contracted at Dum-Dum, and known as kala-azar or Dum-Dum fever. Leishman did not publish his observations until 1903, and in July of that year, Donovan found the same parasite in smears made from material obtained from a splenic punc-



ture made upon a case of kala-azar during life, thus confirming Leishman's discovery. Rogers (1904) was successful in cultivating the parasite and discovered that in cultures flagellated forms developed, while Patton (1907) demonstrated that it also occurred in the peripheral blood and that flagellated forms of the parasite developed in the intestine of insects.

Cathoire (1904) found bodies in the spleen of a child in Tunisia which Laveran diagnosed as *Leishmania donovani*, and Pianese (1905) found the same bodies in the large mononuclear cells in smears from the spleen and liver of children dying from a type of infantile splenic anæmia in Italy, and proposed the term infantile *Leishmania* anæmia to distinguish this condition. Nicolle (1908) observed the parasite in children in Tunis, and proposed the term infantile kala-azar for the disease produced by it. He also proposed the name *Leishmania infantum* for the parasite, believing it to be a new species of *Leishmania*.

Nicolle and Comte (1908) discovered that dogs in Tunis suffered from a form of kala-azar, and that *Leishmania infantum* occurred in the infected animals. Nicolle and his co-workers, especially Basile, have accomplished much very valuable research work upon the subject of infantile kala-azar, the parasite associated with this disease, and the methods of transmission, and believe that the parasite is identical with *Leishmania donovani*, that the naturally infected dog serves as a reservoir of infection for man, and that the parasite is transmitted from dog to man by some insect.

Until very recently, *Leishmania donovani* and *Leishmania infantum* were regarded as distinct species, this opinion being based upon the supposed fact that the latter species caused a disease practically confined to children, and that definite differences existed between *Leishmania donovani* and *Leishmania infantum* in cultures and in the results of animal inoculations. All of these supposed differences have, within recent years, been disproved, and it is now generally accepted that in their morphology, cultural forms, and pathogenic effects the two are alike, and that *Leishmania infantum* should no longer be regarded as a distinct species, but is identical with *Leishmania donovani*. With this opinion I agree, and in this description of the parasite it will be understood that whatever is said regarding *Leishmania donovani* applies also to the species heretofore called *Leishmania infantum*.

Noguchi (1924) has recently shown, by means of monovalent immune serums produced in rabbits by inoculation of cultures of the respective leishmania, that *Leishmania infantum* is identical serologically with *Leishmania donovani*, the anti-donovani and anti-infantum serums agglutinating both organisms.

The exact zoological position of *Leishmania donovani* is still a mat-

ter of controversy, many authorities believing that it should be placed in the genus *Herpetomonas*. However, organisms belonging to the genus *Herpetomonas* are all parasites of invertebrates, principally insects, while the *Leishmania* are parasitic in vertebrates. There is no doubt, in my mind, that *Leishmania donovani* is a *Herpetomonad* which has become adapted to life in the vertebrate host, but, in so doing, changes have occurred in its life-cycle, and even in its morphology, that justify the placing of the organism in another genus. In view of these facts it would seem wise to retain the genus *Leishmania* for the herpetomonads of vertebrates.

**Morphology.**—In describing the morphology of *Leishmania donovani* it is necessary to consider the morphology of the parasite as it occurs in the tissues and blood of man and its morphology as it is observed in cultures.

**Morphology of Parasite in Man.**—*Leishmania donovani* as observed in man consists of a small mass of cytoplasm surrounded by a limiting membrane and containing two clumps of chromatin, one much larger than the other. In living, unstained preparations the morphology of this parasite cannot be distinguished, and staining is always resorted to in diagnosis. Any of the modifications of the Romanowsky stain give good results, but I have obtained the best results with the Wright stain, and the morphology of the parasite in this description is based upon specimens stained with this stain, in both human and cultural forms.

The size of *Leishmania donovani* is variously given by different observers, and the forms observed in the viscera and peripheral blood do vary considerably in size. Castellani and Chalmers (1920) state that it varies from 2 to 3.5 microns in length to 1.5 to 2 microns in breadth, while Archibald (1923) gives the length as from 2 to 4.5 microns and the breadth as from 1 to 2.5 microns. In my experience the length has varied from 2 to 5 microns and the breadth from 1.5 to 3 microns.

The shape of the parasite may be oval, oat-shaped, or spherical, and elongated or torpedo-shaped forms have been described by Knowles (1921). The latter forms are smaller and thinner than the forms usually observed, and are very definitely elongated, having either pointed ends or being sausage-shaped. They have a more definite capsule than the ordinary forms, stain more intensely, and are never intracellular, while the trophonucleus and the blepharoplast are closely associated. I have observed the forms described by Knowles in smears from splenic blood and can confirm his description.

Pyriform organisms are sometimes observed in splenic smears, and at first led to the organism being classed as a piroplasm, but it is thought that such forms are produced by pressure in making the smears.

In fresh unstained material there is never any change in the shape of the parasite, thus proving that it does not possess amœboid motility.

*Leishmania donovani* is surrounded by a limiting membrane which is very delicate and which takes the stain rather poorly. In very deeply stained specimens it may be seen as a bluish membrane surrounding the more dimly stained cytoplasm or shading off gradually into the latter. In many specimens the limiting membrane or capsule is invisible, due to its poor staining qualities.

The cytoplasm stains a dim blue with the Wright's stain and appears homogeneous. In poorly stained preparations the cytoplasm is almost invisible, the only structures visible being the trophonucleus and the blepharoplast. The cytoplasm contains two chromatin masses which stain a pink, red, or lilac, according to the length of time of the staining process. One, the larger, is the macronucleus, or trophonucleus, while the other, which is much smaller, is the blepharoplast, incorrectly called the kintonucleus by many writers.

The macro- or trophonucleus is generally ovoid in shape and stains less intensely than the blepharoplast, taking a dull reddish or pink color with the Wright stain. It is frequently spherical in shape and may be irregular. When oval in shape, it is generally situated at or near the centre of the parasite, but when irregular in shape, it is situated at the periphery of the body. Many parasites show a trophonucleus apparently smeared around a portion of the periphery of the organism, but this appearance is probably caused by pressure in preparing the specimens.

The blepharoplast is very minute in size when compared with the trophonucleus and is rod-like in shape in most parasites, although many are seen in which the blepharoplast consists of a tiny red dot. This body takes the stain very intensely, staining a dark reddish violet or almost black color with the Wright stain. When rod-like in shape the blepharoplast is situated opposite the trophonucleus and generally at a distinct angle to it. When spherical in shape, the blepharoplast is also situated at the opposite side of the body to the trophonucleus, but in neither case is it ever in actual contact with the trophonucleus. However, in the so-called "torpedo-forms" the two bodies often appear fused together because of their close apposition.

In some very well stained specimens there may be observed a prolongation of the chromatic substance of the blepharoplast, extending from it at a right angle as far as the periphery of the parasite, which is called the rhizoplast. This structure is best observed in some of the cultural *Leishmania*.

In spleen smears forms undergoing division may be seen. These are larger than the ordinary forms and show dividing trophonuclei and blepharoplasts and separation of the organism into two daughter

parasites. Parasites are also observed containing numerous trophonuclei and blepharoplasts, and these are believed to be organisms undergoing multiple division or schizogony.

Degenerate forms are frequently observed in smears from the spleen and liver in which the cytoplasm appears much swollen and the macro- or trophonucleus stains poorly or not at all. Such forms are generally filled with small vacuoles and the blepharoplast is the only structure visible that stains normally.

In smears from the liver and spleen of patients suffering from kala-azar the parasites generally occur in groups within the endothelial cells, engulfed in large mononuclear cells, or lying free or in masses in a granular faintly stained matrix, the remains of the degenerated cell in which they have developed. In man, *Leishmania donovani* is an intracellular organism apparently developing within the endothelial cells of the capillaries of the chief viscera and only appears free in the blood plasma or the juice of the organs after it has been liberated by the destruction of the host cell. In the peripheral blood and in smears of the various organs, multitudes of the parasites may be observed within polymorphonuclear and mononuclear leucocytes, and these cells undoubtedly play a very important part in the destruction of the parasites within man. Evidences of degeneration of the parasites are often observed within the polymorphonuclear leucocytes, but it is less certain if the large mononuclear leucocytes destroy them, as the parasites within these cells appear normal and most observers believe that *Leishmania donovani* can live and multiply within these cells.

In the irregular masses of granular substance representing the remains of the cell in which the parasites have developed they may be present in very large numbers, and I have counted over three hundred separate parasites in one such matrix. In this situation degenerated parasites are frequently observed and they are often so crowded together that it is difficult to distinguish their minute structure.

**Morphology of the Parasite in Cultures.**—Leonard Rogers (1904) was the first to successfully cultivate *Leishmania donovani*, and in these cultures found flagellated organisms which, on account of their resemblance to trypanosomes, were so described, and it was not until further culture experiments that it was definitely proven that they were not morphologically identical with any known species of trypanosoma, and were really a stage in the life-history of *Leishmania donovani*.

The first morphological change observed in the parasite in cultures is a considerable enlargement of the trophonucleus, the chromatin at the same time becoming less compact and staining less intensely; coincidentally with the enlargement of the trophonucleus the cytoplasm increases in amount and the entire organism becomes larger, but there is no change



in the size of the blepharoplast throughout the entire process of the development of the flagellated forms. After the changes noted the parasite undergoes division by simple binary fission at least twice, the resulting bodies being round or oval in shape and possessing the same general morphology as the forms observed in man.

After this preliminary division has been completed there appears in the now pyriform body of the organism a small granule which takes a red color with Wright's stain, and which Rogers called the "eosin

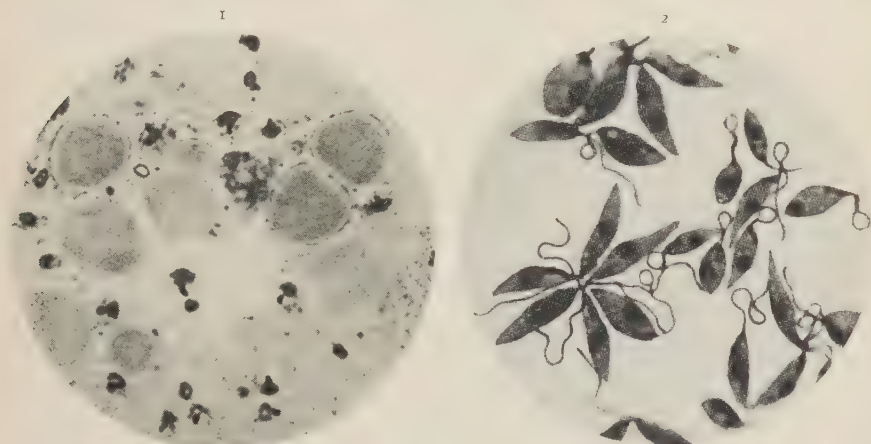


FIG. 58.—*Leishmania donovani*. (Photomicrographs. Army Medical School Collection.) Wright's stain. 1. *Leishmania donovani* in splenic smear from a case of kala-azar.  $\times 1,800$ . 2. Flagellated forms of *Leishmania donovani* from a culture.  $\times 1,800$ .

spot." This granule is closely associated with the blepharoplast and is the basal granule from which arises the flagellum. The parasite now becomes elongated, one end being more narrow than the other, and the blepharoplast and basal granule become situated in the more pointed extremity, in some specimens, and in the rounded extremity in others, the trophonucleus being situated at the opposite end or near the centre of the body.

After the blepharoplast and basal granule have become situated in the end of the body the basal granule gives rise to a long, very delicate flagellum which projects from the body and at once becomes a free flagellum. In most specimens stained with Wright's stain the flagellum appears to originate directly from the blepharoplast due to the close association of the basal granule with the latter and to diffuse staining, but with wet-fixation and hæmatoxylin staining all of the structures mentioned may be distinguished. No undulating membrane is present in the flagellated forms.

After the development of the flagellum the entire organism elongates greatly, the extremity farthest removed from the blepharoplast becom-

ing narrow and pointed, while that from which the flagellum arises may be more rounded in appearance, but in the fully grown flagellates both extremities are often pointed. The trophonucleus is generally central in position, but may be situated toward the non-flagellated extremity.

In cultures all the forms that have been described may be seen, from the forms having the morphology of those occurring in man to the fully developed flagellate, provided the culture is not too old. In old cultures the parasites lose their flagella and apparently revert to the oval and spherical forms found in man, but having a thicker capsule. These forms will again develop flagellate forms if placed in fresh culture media, and some authorities regard them as resting or cystic stages of the parasite.

Flagellated forms of *Leishmania donovani* have never been found in the human host, but according to the observations of Patton and others, occur in the intestine of bed-bugs that have fed upon patients suffering from kala-azar.

Dividing forms of the flagellate stage of *Leishmania donovani* are common in cultures, division occurring longitudinally. The forms resulting from equal longitudinal division are at first pear-shaped but soon elongate and become actively motile, much more so than were the parent bodies at the time of division. In these parasites the basal granule from which arises the flagellum is situated anteriorly, while the trophonucleus is at or near the centre of the body.

Division of the parasite is preceded by the binary division of the blepharoplast, basal granule, and flagellum, followed by the division of the trophonucleus, after which there is a longitudinal splitting of the whole body into two portions, each of which repeats the process of division within a short time. This rapid division results in the formation of the so-called "multiplication rosettes," in which the flagella all point toward the centre. In these rosettes the parasites may vary considerably in morphology, pear-shaped forms and elongate forms occurring in the same rosette.

Leishman (1905) describes a process of multiplication in the flagellate forms of *Leishmania donovani* in which multiple spirilla-like forms are split off longitudinally from the parent body, and in cultures I have frequently seen organisms apparently undergoing this form of division. In my experience such forms have occurred in older cultures.

The size of the cultural forms of *Leishmania donovani* varies greatly during the different stages in the development of the organism. Forms occur that are similar in size to those observed in man, but the fully developed flagellate form measures from 15 to 25 microns in length and from 1.5 to 3.5 microns in breadth. The flagellum is very delicate and measures from 15 to as much as 28 microns in length.

**Habitat.**—*Leishmania donovani* is found within the endothelial cells of the capillaries of nearly every organ of the body of man. In these cells they live and multiply, gradually destroying them, and when this occurs the liberated parasites are phagocyted by the large mononuclear and polymorphonuclear leucocytes. They are most numerous within the endothelial cells of the capillaries of the spleen, liver, bone marrow, intestinal mucosa, and mesenteric glands, but are also found, in severe infections, in the endothelial cells of the kidneys, suprarenal capsules, lungs, meningeal vessels, and in the cerebrospinal fluid.

Not only do the endothelial cells of the capillaries show the parasites, but they may lie free within the capillaries or be found within the large mononuclear and polymorphonuclear cells, many of the capillaries being entirely blocked by accumulations of parasites and cells containing them.

The peripheral blood, in a considerable proportion of infections with *Leishmania donovani*, contains the parasites either engulfed in the large mononuclear and polymorphonuclear leucocytes or lying free in the plasma. The observation so often repeated in text-books, that *Leishmania donovani* is found within the red blood corpuscles, is erroneous, as this parasite never occurs in that situation.

In the ulcerations so frequently found in the large intestine in kala-azar the parasites may be demonstrated in smears, lying in endothelial cells, large mononuclear cells, and polymorphonuclear leucocytes. In some infections the cells of the liver and spleen have been found invaded, but only in small numbers. Many of the large mononuclear cells lying free in the capillaries are endothelial in origin and are usually packed with *Leishmania*.

**Species Occurring in Lower Animals.**—So far as has been determined the only species of lower animal that is naturally infected with *Leishmania* is the dog. That these animals are infected with a parasite indistinguishable, morphologically, culturally, and in results obtained by animal experimentation, from *Leishmania donovani* has been proven by Nicolle and Comte (1908), Nicolle and Basile (1909), and others, and while these facts do not prove that the parasite of the dog is identical with *Leishmania donovani*, it is quite generally accepted that the two species are identical. Nicolle and Basile, who have studied very exhaustively the natural infection in the dog and experimental infections in these animals produced by the injection of *Leishmania donovani*, conclude that the symptoms and pathological lesions produced in both infections are identical, and this would appear to be good evidence that the dog is a host of *Leishmania donovani* and that the disease known as canine Leishmaniasis is caused by this parasite.

The question of the occurrence of *Leishmania donovani* in insects

brings up the entire subject of the method of transmission of this infection and will be considered later. (See Method of Transmission.)

**Cultivation.**—Rogers (1904) successfully cultivated *Leishmania donovani* by placing material obtained from splenic punctures in sodium citrate solution and keeping the mixture at 22° C. In these cultures the typical flagellate forms developed which he described and which differ so markedly from the forms of the parasite which are found in the human body. Rogers' results were soon confirmed by a host of observers and his method of cultivation greatly improved upon by the use of a mixture of agar and defibrinated rabbit's blood, the so-called Novy-MacNeal, Nicolle, or N.N.N. medium. (See Appendix.) Tubes of this medium are prepared and the water of condensation inoculated with blood or material from splenic or liver punctures. In the water of condensation the *Leishmania* develop into flagellated forms very readily if the tubes are kept at 22° C. and do not become infected with bacteria. If bacteria develop the *Leishmania* do not multiply and disappear rapidly. The range of temperature for successful cultures is between 20° and 22° C.

The flagellate forms begin to appear in the cultures at about the third day and their morphology has already been described. In old cultures the parasites lose their flagella and become oval or spherical in shape, but if reinoculated into fresh media, such forms again become flagellated and typical in appearance of the cultural forms of *Leishmania donovani*.

The *Leishmania* parasitic in the dog is easily cultivated upon the N.N.N. medium and develops flagellated forms indistinguishable morphologically from those of *Leishmania donovani*. Such cultures, when inoculated into animals that are susceptible to infection with *Leishmania donovani*, will cause infection with the production of the same symptoms and pathological lesions as follow inoculation with the latter parasite, thus apparently demonstrating the identity of the two organisms.

The use of cultures in the diagnosis of infection with *Leishmania* is most important and will be discussed in the consideration of the diagnosis of these infections.

**Life-history.**—The life-cycle of *Leishmania donovani* has not been demonstrated in its entirety, and our knowledge of even the life-cycle in the human host is still regarded as incomplete by many excellent authorities. So far as the human life-cycle is concerned we know that *Leishmania donovani* lives and develops within the endothelial cells and, apparently, within the large mononuclear leucocytes, while rarely, it is found within the tissue cells of the invaded organs of the human body. In these cells the organism multiplies by simple binary fission, the multiplication continuing until the host cell is entirely filled, and eventually destroyed, at which time the parasites are liberated. New cells are then



invaded and the process of multiplication and ultimate destruction of the host cell is repeated. Multiple division within the host cells is believed to occur by many authorities, the process being called schizogony. Resistant or encysted forms of the parasite have been described as developing in the spleen and other viscera, but there is no general agreement as to the nature of these forms, and many observers deny their existence.

That *Leishmania donovani* is capable of developing in the intestinal canal of the bed-bug was first proven by Patton (1907), who observed in *Cimex hemiptera*, that had fed upon patients suffering from kala-azar, forms of the parasite identical with those observed in cultures. His observation has been confirmed by many investigators, and Cornwall and La Frenais (1916) showed that the flagellate forms so developed may remain alive in the intestine of the bug for twenty-nine days, while Patton, La Frenais, and Rao (1921) obtained living *Leishmania donovani* from the mid-gut of the bed-bug forty-one days after the insect had fed on cultures of the parasite.

Cornwall and La Frenais (1916) described a peculiar form of *Leishmania donovani* in the intestine of the infected bed-bug, which they called the "thick tail" flagellate. This form is spherical in shape, from 5 to 6 microns in diameter, and has a flagellum four or five times as thick as the flagellum of the ordinary flagellated form of *Leishmania*. The significance of this form in the life-history of the parasite they did not determine.

Adie (1922) found that *Leishmania donovani*, during its development in the bed-bug, lives not only in the gut cavity but invades the cells of the mucous membrane, and it is the "thick tail" flagellated form that is believed by Adie to invade these cells and develop into a sporulating parasite. Cornwall and La Frenais (1921) could find no evidence in the material they studied that supported Adie's descriptions and conclusions and state that the "thick tail" flagellate "is clearly an encapsulated flagellate," concluding that it may be a necessary phase in the development in the true insect host, which they do not think has been discovered. They also call attention to the fact that the "thick tail" forms are very rare, thousands of the ordinary flagellated forms being observed in the intestine of the bug to one of the "thick tail" flagellates.

Patton (1922) states that he has confirmed Adie's discovery of the intracellular stage of *Leishmania donovani* in the mid-gut of the bed-bug (*Cimex hemiptera*), and he regards this fact as proving conclusively that the bed-bug is the true invertebrate host of *Leishmania donovani*.

The question of the occurrence of *Leishmania donovani* in the salivary glands of the bed-bug has, until recently, been answered in the negative. However, in November, 1921, Adie announced the discovery of forms of *Leishmania donovani* in the salivary glands and ducts of a

bed-bug caught upon the bed of a suspected case of kala-azar, and if her discovery is confirmed, a new chapter will have been opened in the life-history of this parasite in the bed-bug, but already, Wenyon (1922) states that the structures that Adie observed in the salivary glands and ducts of the bug were "neither Leishman-Donovan bodies nor stages of any other flagellate," according to reliable information received from India.

From what has been said it is evident that the life-history of *Leishmania donovani* is still unknown in many of its most important aspects and that while there is no doubt that the parasite can develop in the bed-bug, the question of the significance of this stage in its life-history is still undecided.

The development of *Leishmania donovani* in the flea, which has been so graphically described by Basile and others, is still open to question. Basile (1920) claims that development of cystic forms of the parasite occurs in the hind-gut of the flea, but his observations await confirmation. Da Silva (1913), Wenyon (1914), and Patton (1921) have all failed to find any evidence of a life-cycle in the flea and have been unsuccessful in obtaining any development of *Leishmania donovani* in this insect.

Knowles, Napier, and Smith (1924) have found a *Leishmania* in the gut of *Phlebotomus argentipes* feeding upon kala-azar patients, and Christophers, Shortt, and Barrand confirmed their findings in laboratory-bred *P. argentipes* allowed to suck the blood of kala-azar patients. All of these observers agree as to the identity of the *Leishmania*, and further observations may show that this insect is a transmitter of this parasite.

**Geographical Distribution.**—The geographical distribution of *Leishmania donovani* is confined to Southern Europe, Asia, and Africa. It has not been demonstrated in North or South America or any of the islands of the Atlantic, Pacific, or Indian Ocean.

In Europe the worst endemic areas are in Italy, Sicily, Crete, Greece, and Malta. In Italy the endemic areas are in the southern portion bordering on the Mediterranean Sea. A few cases of infection have been found in Spain and Portugal.

In Asia the endemic centres are in India and Assam. In India the infection is endemic along the Ganges and Brahmaputra Rivers, the deltas of these rivers being badly infected, and in Madras. Isolated cases also occur in Ceylon, Turkestan, Burma, Syria, and Indo-China. Endemic infection exists in Central China, Pekin, and the valley of the Yang-tse-Kiang.

In Africa, *Leishmania donovani* is common in Tunis, Tripoli, and Algeria, while Archibald reports infections in the Sudan with a variety of the parasite which is probably identical with *Leishmania donovani*.

Isolated infections have been reported from Egypt, Khartoum, and French Guinea.

While, at the present time, the geographical distribution of this parasite is limited to the countries mentioned, there would appear to be no good reason why, with the constantly increasing facilities for rapid communication between the countries of the world, it should not eventually appear in regions that are now free from infection. Patients suffering from continued fever accompanied by enlargement of the spleen and coming from the infected regions should always be regarded with suspicion and carefully investigated.

No less than ten cases of infection with this parasite have already been described in the United States by different observers. The cases have all been imported ones up to date, but it is not at all improbable that the infection may, in the future, become endemic in certain regions where favorable climatic conditions and the presence of the transmitting agent are combined and unrecognized cases are imported. Imported cases of infection with *Leishmania donovani* in the United States have been described by Faber and Schusler (1923), Spencer (1921), Smith (1922), Fox (1922), and Lambert (1923).

**Incidence of Infection.**—The numerical incidence of infection with *Leishmania donovani* varies greatly in different localities. In the most badly infected regions, as the Garo Hills of Assam, and in the low-lying regions along the Ganges, a very considerable proportion of the natives suffered from the infection during epidemics, while in Ceylon, Egypt, Spain, and Portugal, only isolated instances of infection have been observed. The incidence of infection in certain regions in India, as Madras, is high, the infection showing the symptomatology of typical kala-azar, while in the Mediterranean region the incidence is high in Tunis, Tripoli, and Algeria, and in some parts of Italy and Sicily, and in these regions the infection presents the symptomatology of infantile splenic anæmia or infantile kala-azar. In the Mediterranean region *Leishmania donovani* most frequently attacks infants from one to four years of age, and adults are seldom found infected; in India young adults are most frequently found infected, but the infection may occur at any age; while in the Sudan, Archibald (1923) states that infection occurs most frequently in late childhood and early adult life. The differences in the age incidence mentioned was long held as a powerful argument for the specific status of *Leishmania infantum*, but recent studies have shown that while there is a preference in different localities as regards age, infections in all localities may occur either in infancy or adult life, and it is no longer held that the age incidence proves the non-identity of *Leishmania donovani* and *Leishmania infantum*.

*Leishmania donovani* attacks both sexes, but males are more fre-

quently infected than females. Natives appear to be more frequently attacked than Europeans in India but, as Archibald states, the great disproportion in numbers between them and the natives should be remembered. Infection is most common in those living in unhygienic surroundings, and moisture and a moderate degree of heat appear to favor its spread in epidemic form. It is much more frequently an infection of rural districts than of cities and of debilitated and under-nourished individuals than of the healthy and robust. Family infection is often noted and certain houses appear to retain the infection for long periods of time.

**Method of Transmission.**—The method of transmission of *Leishmania donovani* from man to man is unknown. An infected locality, close personal association between the sick and the well, and prolonged exposure to the infecting agency, probably an insect, appear to be necessary for the transmission of the infection. The rôle played by the human "carrier" of *Leishmania donovani* in the transmission of the parasite is at present unknown, but it is probable that "carriers" are very important in this respect.

There are two principal theories as to the method of transmission of *Leishmania donovani*, the first, and most probable, being that it is transmitted from man to man by some biting insect, and the second, that the parasite is ingested in contaminated food or drink.

**Transmission by Insects.**—The fact that *Leishmania donovani* develops readily into flagellate forms in cultures kept between 20° and 22° C. suggested that similar forms might develop in the intestinal canal of some insect and that these might be the infecting forms for man, and the insect the transmitting agent.

Patton (1907) discovered flagellate forms of *Leishmania donovani* in the stomach and intestine of the bed-bug (*Cimex hemiptera*) after feeding upon the peripheral blood of patients suffering from kala-azar, and concluded, from his results, that this insect is the transmitting agent in India. In 1921, Patton published a paper giving the results of his work upon the insects reputed by various observers to be concerned in the transmission of *Leishmania donovani*, which include mosquitoes, sand-flies, fleas, lice, ticks, and bugs. The following data regarding the transmission of the parasite by these insects is taken largely from Patton's paper and the papers of the investigators referred to in the discussion that follows.

**Mosquitoes.** Patton has not been able to find the flagellate stage of *Leishmania donovani* in any mosquito that he has examined and calls attention to the fact that these insects are infected with *Herpetomonads* of their own, and that these have probably been mistaken for the flagellate stage of *Leishmania donovani*. In addition, the epidemiological evidence is against a flying insect being the intermediate host of this



parasite, which causes a localized infection, as at George Town, Madras, where kala-azar is confined to certain streets and even to certain groups of houses. To my mind this fact is absolutely conclusive that mosquitoes do not transmit *Leishmania donovani*. There is no evidence at present that these insects ever transmit this parasite.

*Sand-flies.* Laveran and Franchini (1919) claim to have produced a disease in dogs indistinguishable from kala-azar by infecting them with a species of *Herpetomonad* parasitic in *Phlebotomus papatasi*, but their observations have not been confirmed. Mackie (1915), Hoare (1921), and others have repeated the work of Laveran and Franchini with absolutely negative results.

*Fleas.* Basile (1916-1920) has studied the question of the transmission of *Leishmania donovani* by the flea and believes that this insect is the transmitting agent of the parasite in the Mediterranean region. He states that although the flea is parasitized by other flagellates it is possible to distinguish them from the flagellate stage of *Leishmania donovani* and that the latter parasite develops into flagellate forms in the intestinal tract of the flea. He also states that the flagellates encyst in the hind-gut of the flea and that man may be infected either through the bite of the insect or by ingesting material containing these cystic forms.

Patton (1922) has never been able to trace any definite continued development of *Leishmania donovani* in the flea. He states that he has fed *Pulex irritans* with cultures rich in *Leishmania donovani* but, beyond finding the flagellates in the stomach twenty-four hours later, no development occurred and the flagellates soon perished. Wenyon (1914) also failed to obtain any development of *Leishmania donovani* in the flea and was unable to transmit the infection with these insects.

The work of Basile is, at the present time, regarded with skepticism and there is no scientific proof available that the flea is the transmitting agent of *Leishmania donovani*.

*Lice.* Patton (1921) was unable to find any flagellate forms of *Leishmania donovani* in the intestinal tract of either the head or body louse after feeding upon patients suffering from kala-azar. The usual forms observed in man occurred in the stomach of lice but no development into flagellates followed and the organisms rapidly disappeared.

*Ticks.* There is no real evidence that any species of tick is concerned in the transmission of *Leishmania donovani*. Patton (1921) states that he has fed *Ornithodoros savignyi*, the only tick that fed on man in the locality in which he worked, on patients whose peripheral blood contained *Leishmania donovani*, but never observed any developmental forms of the parasite in the tick. Similar experiments have been tried with *Ornithodoros moubata* with negative results.

*Bugs.* The genus *Triatoma* (*Conorhinus*) contains species which have been suspected of being the invertebrate host of *Leishmania donovani*, and Patton has investigated this subject very thoroughly. He found that *Triatoma rubrofasciatus*, when fed upon kala-azar patients, does not show any development of the flagellate stage in its intestinal tract, and that the ingested *Leishmania* degenerate and disappear in a short time after the feeding. His observations have confirmed the previous results of Cornwall and La Frenais (1916).

*Bed-bugs.* The bed-bug, *Cimex hemiptera*, has been found by Patton and numerous other observers to be an efficient host for *Leishmania donovani*, so far as the development of the flagellate stage is concerned. There is no question that in the intestinal tract of this bug, if fed upon patients whose peripheral blood contains *Leishmania donovani*, there develop flagellate forms of the parasite identical with those observed in cultures, but though this is true, the proof that the bed-bug is the natural invertebrate host of the parasite, and that it is the transmitting agent of the parasite from man to man, is still lacking, in the opinion of the best students of the subject.

Patton's researches upon the development of *Leishmania donovani* in the bed-bug have extended over many years and are deserving of the most careful consideration. Patton (1921) states that he first noted the flagellate stage of *Leishmania donovani* in the intestinal tract of bed-bugs (*Cimex hemiptera*), that had bitten patients suffering from kala-azar, in 1905, and his observation has since been confirmed by many investigators. He found that as soon as the cells containing the *Leishmania* were digested they were liberated, and immediately developed into flagellates. If the bugs are not fed again the flagellate forms multiply and eventually become round forms again. If the bed-bug is fed upon cultures of *Leishmania donovani* containing living flagellates and the contents of the gut of the insect be cultured repeatedly, the flagellates are found in a living condition in the mid-gut for a period of thirty-one days after feeding, and in the hind-gut and rectum for as long as thirty-four days after feeding. If the bugs, after feeding upon the cultures, were refed upon uninfected human blood, the flagellates could be found in the mid-gut for forty-one days after feeding upon the cultures, and in the hind-gut and rectum for thirty-four days. He also noted that only a very small proportion of the bed-bugs that fed upon the cultures, or upon individuals whose peripheral blood contained numerous *Leishmania donovani*, became infected, not over 5 per cent. in his experience.

As regards the question of how the bed-bug transmits the infection to man, Patton (1921) states that he has never found the flagellate stage of *Leishmania donovani* in the salivary glands or in the pumping organ

of the bug, and that infection of man does not occur through the salivary secretion injected into the wound at the time of biting or by regurgitation when the bug sucks blood. In his opinion the infection is transmitted by crushing the bug upon the skin while it is biting, when the flagellates in the intestinal tract are rubbed into the wound caused by the bite of the insect or into abrasions of the skin produced by scratching.

Patton believes that his observations upon the transmission of *Leishmania donovani* by the bed-bug explain many of the peculiar epidemiological facts connected with the spread of kala-azar in infected districts. The localized character of the disease, even to the extent of definite house infections, is explained by the habits of the bed-bug, which localizes itself in houses and does not travel for any great distance, and by the fact that only a very small proportion of bugs that bite individuals with *Leishmania donovani* in their peripheral blood become infected, and the chances of the small number becoming infected being transported to any great distance being so small. For these reasons kala-azar spreads very slowly from the endemic areas, but that it does spread is evidenced by the fact that new endemic areas are constantly being established.

Although Patton's theory regarding the relation of the bed-bug to the transmission of kala-azar to man is most plausible and probable, the final proof of its truth, *i.e.*, the actual experimental transmission of *Leishmania donovani* from the bug to man or lower animals, susceptible to infection with it, has not been furnished, all attempts in this direction having been unsuccessful. However, the epidemiology of kala-azar and the results of his researches, and those of others, all point to an insect as being the transmitting agent of this infection, and of the insects, so far investigated, the bed-bug appears to be the most probable agent of transmission.

**Transmission through the Alimentary Tract.**—Many patients suffering from infection with *Leishmania donovani* present symptoms of severe and even fatal diarrhoea or dysentery and, in the fatal cases, the intestinal mucous membrane of the large intestine, especially, is often greatly inflamed and presents ulcerations. In the walls of the ulcers *Leishmania donovani* is found in large numbers lying within the endothelial and large mononuclear cells and phagocyted within leucocytes. This fact has led some authorities to believe that the parasites may pass from the inflamed areas and ulcers into the lumen of the intestine, whence they may be passed with the faeces in some resistant stage or cyst, which can withstand external influences. These forms, according to the proponents of infection through the alimentary tract, may



contaminate food or drink and thus reach man, developing into the typical forms in the intestine.

Although diligent search has been made for them, by many competent observers, leishmania have never been found in the fæces, and Patton (1921) states that the bloody mucus from the stools of kala-azar patients, when kept under the most favorable conditions for the development of the flagellated forms, has always given negative results. If the parasites were present in this material it is almost impossible to believe that they would not develop in cultures made from it upon suitable media, but such cultures have never been successful.

Fantham and Porter (1916) have been successful in infecting certain laboratory animals with the *Herpetomonad* of the flea by feeding them infected insects, but Patton (1921) states he was unsuccessful in producing infection in monkeys (*Macacus sinicus*) by feeding them mucus from the intestine of patients suffering from kala-azar or splenic juice rich in *Leishmania donovani*. Archibald (1923) states that, in the Sudan, carefully conducted feeding experiments with material containing *Leishmania donovani* were successful in producing infection in monkeys, and that similar results were obtained by Basile in Europe. Further observations are needed before one can accept the results of the feeding experiments recorded, as they were open to criticism from the standpoint of technique and controls.

There is not, at present, any evidence of scientific value supporting the theory that the transmission of *Leishmania donovani* from man to man is due to food or drink contaminated by forms of the parasite that have been voided in the fæces of kala-azar patients.

Shortt (1923) has recorded the finding of *Leishmania donovani* in the urine of a patient observed in Shillong. Cultures were made from material obtained by liver puncture, and an abundant growth of *Leishmania donovani* obtained. The urine was then collected under aseptic precautions and cultures made from it resulted in a growth of *Leishmania donovani*. This strain, in subcultures, was found to be identical with that obtained from the blood of kala-azar patients. In a later communication, Shortt, Swaminath, and Sen (1923) report the successful cultivation of *Leishmania donovani* in three of nine cases of kala-azar by adding the sediment of the urine, collected aseptically, to tubes of N.N.N. medium, the parasites in the cultures being identical with other strains of *Leishmania donovani*.

These observations demonstrate that the urine may contain *Leishmania donovani* in patients suffering from infection with this parasite, and further observations will undoubtedly solve the question of the relation of this fact to the transmission of the infection.

Another theory of transmission that has been advocated is that



forms of *Leishmania donovani* passed in the fæces may be taken up by some insect in which development occurs and that this insect may transmit the infection. This theory has still less evidence in its favor than the theory of the direct transmission of the parasite to man through contaminated food or drink. The same may be said of the theory of the transmission of the parasite by *Helminthes*, especially the hook-worm.

**The Dog as a Reservoir of Infection.**—In the Mediterranean region it has been demonstrated that dogs are naturally infected with a *Leishmania* that morphologically, culturally, and experimentally is indistinguishable from *Leishmania donovani*, and that produces in the infected animals symptoms and lesions similar to those of kala-azar in man. These facts have led many observers to believe that the dog is a natural reservoir of infection for man, and that the flea is the transmitting agent. The dog fleas, *Pulex serraticiceps* and *Pulex canis*, have been incriminated by Nicolle and Basile, but their observations have not been confirmed by others, and the fact that canine leishmaniasis is unknown in the great endemic areas of kala-azar in India, Assam, and in the Sudan is evidence that the dog is not necessary for the perpetuation of this infection, and it is very doubtful if it acts as a reservoir of infection even in the regions where canine leishmaniasis is common.

**Experimental Infection of Lower Animals.**—The only animal that suffers naturally from infection with *Leishmania* is the dog, but other animals can be infected experimentally, although with difficulty in most instances. Several of the lower animals are susceptible to experimental infection with *Leishmania donovani*, and for a while it was believed that the differences shown in susceptibility to various strains of the organism might be of service in differentiating them. Thus it was found that slight differences existed in the ease with which some animals became infected with *Leishmania donovani* from the Mediterranean region, India, and the Sudan, but it is now generally admitted that these differences are so slight as to be of no service in supporting the theory that the *Leishmania* from these different regions are distinct species or even varieties. Novy (1908) was the first to produce infection in dogs with cultures of *Leishmania infantum* (*Leishmania donovani*).

*Leishmania donovani*, of the Mediterranean region, has been successfully transmitted experimentally to white mice, rats, guinea-pigs, rabbits, the jackal, dogs, and monkeys. Large doses of the virus are necessary to infect guinea-pigs and rabbits, and intraperitoneal injection is most successful.

*Leishmania donovani*, as encountered in India, has been experimentally transmitted to the white mouse, white rat, flying fox, jackal, dog, and monkey, but guinea-pigs and rabbits are apparently immune.

*Leishmania donovani*, of the Sudan, has been experimentally trans-

mitted to the dog, gerboa, gerbil, and monkey. Guinea-pigs, rabbits, white mice, and rats are apparently immune. The gray monkey has been infected by feeding it emulsions of the Sudan virus.

It will be noted that there are differences in the transmissibility of *Leishmania donovani* from the regions mentioned above to certain of the lower animals, but these differences are not believed to be sufficient to indicate that there are three species of *Leishmania* concerned, and Laveran has proven that *Macacus* monkeys rendered immune to infection with *Leishmania donovani* from the Mediterranean region are also immune to the *Leishmania donovani* of India.

**Relation to Disease.**—*Leishmania donovani* is the cause of an acute, subacute, or chronic infective disease, occurring in children and adults, characterized by enlargement of the liver and spleen, irregular fever, progressive anæmia, and a high mortality. The organism is always found in the affected viscera, lymphatic glands, and bone marrow, and is not found in health or in any other disease condition. The inoculation of *Leishmania donovani* into suitable animals causes lesions similar to those occurring in man, and the organism can again be recovered from these lesions in cultures.

The condition produced by infection with *Leishmania donovani* in the Mediterranean region is known as infantile splenic anæmia, or infantile splenomegaly, while in India the condition is known as kala-azar, and for a long time it was thought that the two were distinct and caused by distinct species of *Leishmania*. However, recent research has shown that adult infections also occur in the Mediterranean region and infantile infections in India, and as the parasites occurring in both places are indistinguishable morphologically, culturally, and in their pathogenic effects upon susceptible lower animals, it is no longer believed that there is any specific difference in the parasites found in the two regions mentioned or in the Sudan. The name, *Leishmania infantum*, was given by Nicolle, in 1908, to the parasite found in infantile splenic anæmia, but it is now regarded by the best authorities as merely a synonym of *Leishmania donovani*.

The lesions produced by *Leishmania donovani* consist of marked hypertrophy of the spleen to such an extent that it may sometimes be palpated some distance below the umbilicus; enlargement of the liver; congestion and ulceration of the large intestine; enlargements of the lymphatic glands; and changes in the blood-forming organs producing a progressive and severe anæmia, most evident in the infantile infections.

As already mentioned, the parasites are found in the endothelial cells of the capillaries of the infected viscera or within large mononuclear and polymorphonuclear leucocytes within the blood-vessels. They may also be found in the eosinophilic leucocytes, rarely within the cells

of the parenchyma of the invaded viscera, or lying free in the blood plasma. In the spleen they occur in immense numbers and are also very numerous in the bone-marrow and liver.

There is no sufficient evidence proving that recovery from an infection with *Leishmania donovani* confers any immunity, and while agglutinins and precipitins have been demonstrated in the blood of some cases, they have been present in small amounts and for a limited period of time.

**Prophylaxis.**—The prophylaxis of infection with *Leishmania donovani* is empirical, as we possess no definite knowledge of the method of transmission. The rôle of the "carrier" in this disease is probably of great importance, as certain individuals suffer from the infection for long periods of time but are able to keep about and attend to their duties, and it is probable that these individuals are responsible for a considerable amount of the infection in endemic regions. The discovery of such "carriers" is, therefore, an important prophylactic measure.

The isolation of infected individuals should always be insisted upon, and while there is no real evidence that the discharge from the bowel transmits the infection, it is the part of wisdom to disinfect the stools. As the infection appears to be localized in certain houses in the endemic regions, such houses should be disinfected or destroyed, and immigration between infected and uninfected regions should be carefully controlled. Measures directed toward the destruction of insects, especially the bed-bug, should be taken, for there is enough evidence available to indicate that some insect is the transmitting agent of the infection.

The fact that dogs are naturally infected with a parasite indistinguishable from *Leishmania donovani*, and that these animals can be experimentally infected from man, indicates that measures should be taken to properly control them. Close contact between man and dogs in the endemic regions should be avoided as much as possible, and as canine leishmaniasis is prevalent in those regions in which the infantile type of kala-azar is encountered, special precautions should be taken to keep children apart from these animals.

Infection with *Leishmania donovani* is most frequently observed in individuals living in unhygienic surroundings, and attention to personal hygiene is a most important prophylactic measure. Cleanliness in person and surroundings will generally result in improved conditions as regards the prevalence of insects and thus aid greatly in the prophylaxis of the infection.

**Diagnosis of Infection with *Leishmania donovani*.**—The diagnosis of infection with *Leishmania donovani*, or kala-azar, rests upon the demonstration of the presence in the body of the suspected individual of this parasite. The diagnostic methods that have been, or are, employed include splenic puncture, with or without subsequent culture;



direct examination of the peripheral blood; culture of the peripheral blood; animal inoculation of the peripheral blood; examination of the lymphatic glands; and serum tests.

For the demonstration of *Leishmania donovani* staining methods must be employed, and the most generally useful for diagnostic purposes are the Wright or Leishman stain. The material to be examined should be smeared upon a clean microscopic slide, allowed to dry, and then stained. I have found that the Wright stain gives me the best results, the preparation being covered with the stain, which is allowed to remain for two or three minutes, in order to fix the specimen, before adding freshly distilled water. The water should be added until a well-marked metallic film appears upon the surface of the mixture, and then the specimen is allowed to stain for from five to ten minutes, or even longer, according to the intensity of the stain desired. The specimen is then washed thoroughly in distilled water, dried, and examined directly, using an immersion lens. If desired, the preparation may be mounted with a cover-glass in neutral balsam, but this is unnecessary as preparations keep better if not mounted. After examination is completed the immersion oil should be washed off carefully with zylene, if it is desired to keep the preparation, and the slide placed in a light-proof box or slide cabinet. When properly stained the cytoplasm of the parasite takes a pale-blue color, the nucleus a rose-pink or bright-red color, and the blepharoplast and basal granule a very dark red or violet color. In the flagellated forms the same staining reactions occur and the flagella are stained a pink or reddish color.

**Diagnosis by Splenic Puncture.**—Patients suffering from infection with *Leishmania donovani* have an enlarged spleen which is generally easily palpable, and the puncture of this organ, and the examination of the material so obtained, still remains the most rapid and successful of the diagnostic methods which have been devised. Although this is true, splenic puncture is not devoid of danger, and should not be employed until other methods of diagnosis have proven negative.

The first, and a most essential, step in the technique of splenic puncture, is the determination of the coagulation time of the blood of the patient and this is best accomplished by the use of the Wright tube. One cubic centimetre of blood is withdrawn from a vein of the arm with a small hypodermic syringe which has been carefully sterilized, the time of withdrawal is noted, and the blood placed immediately in a glass tube of 8 mm. diameter which has been rinsed in normal saline solution. This tube is then rotated endwise every thirty seconds, and the time accurately noted when the blood no longer flows, but remains in position. The normal coagulation time, as determined in this manner, is from five



to eight minutes, but most authorities do not advise splenic puncture if the coagulation time is more than five minutes.

The second step preparatory to puncture is the administration of calcium chloride in order to lessen the chances of hemorrhage. Twenty grains of calcium chloride are given the night before the day upon which puncture is to be made, the same dose repeated two hours before the puncture, and immediately after the puncture has been completed.

The patient should be in bed and an abdominal binder should be in readiness beneath the body to immobilize it as much as possible after the puncture is completed. The patient should be warned to take a deep breath before the puncture is made, and to hold it, and to avoid making any sudden movement when the needle is plunged into the spleen, in order to obviate danger of tearing the capsule of the organ. The skin over the site of the puncture should be sterilized with tincture of iodine and the syringe and needle used in the operation should also be sterilized. The syringe employed should be a 5 c.c. glass syringe fitted with a very sharp needle. A sterile gauze pad should be at hand to cover the wound made by the needle. The needle should be inserted quickly and steadily into the spleen at right angles and the syringe held loosely so that if the patient jerks away during the penetration of the needle there will be less chance of tearing the spleen. After insertion, slow, gentle suction is made with the piston of the syringe and as soon as blood-stained material begins to appear in the syringe, the needle is withdrawn and the material at once placed upon microscopic slides or distributed to culture tubes. As soon as the needle is withdrawn the sterile gauze pad is placed over the puncture and retained by the bandage and the patient kept in bed as quiet as possible for at least twenty-four hours. If the first puncture does not result in securing material it may be repeated, or, as Knowles recommends, the needle may be very slightly withdrawn and passed again into the splenic pulp in another direction. If blood enters the syringe at once, and in some volume, it indicates that a splenic vein has been entered and it is best to withdraw the needle and empty the syringe, as few parasites are found in such material.

It should be remembered that very little splenic juice is needed in order to demonstrate *Leishmania donovani*, and it is a mistake to endeavor to obtain more than from one-half to one c.c. of material by splenic puncture. In fact, a diagnosis may be made from a very little material in advanced cases of infection, one or two drops of splenic juice being sufficient.

Puncture of the liver is a safer procedure than splenic puncture, and is performed in the same manner. It should always be considered in lieu of puncture of the spleen if conditions are such as to make the latter method inadvisable. The puncture of the liver is made in the

eighth intercostal space in the anterior axillary line. Puncture of the liver is not as sure a method of diagnosis as puncture of the spleen, but is often successful. Either method, if properly performed, is practically devoid of danger to life, but deaths have occurred from hemorrhage after spleen puncture in cases in which the coagulation time of the blood had not been ascertained, and calcium chloride had not been administered.

*Diagnostic Results of Splenic Puncture.* Examinations of material obtained by splenic puncture in cases of infection with *Leishmania donovani* give a very high percentage of positive results, ranging from 90 to 95 per cent. or more, and if, in addition, the material be cultured on N.N.N. medium, 100 per cent. of positive results may be expected. It should be remembered, however, that enlargement of the spleen, due to other conditions, is common in many localities where kala-azar is endemic, and a negative result from splenic puncture is sometimes obtained, for this reason, in cases that are regarded clinically as very suspicious. Thus, Nicolle (1921), in the examination by splenic puncture of 172 suspicious cases, obtained positive results in only 59 cases, but in none of the negative cases could the suspicion of the presence of *Leishmania donovani* be confirmed.

Knowles (1920) obtained 76 positive results by splenic puncture and culture of splenic juice obtained by puncture, in 79 cases of kala-azar, or 96 per cent. The positive results were divided as follows as regards the technique employed:

Stained films of splenic juice were positive in 21 cases; both stained films and cultures on N.N.N. medium were positive in 47 cases; stained films positive and cultures negative in 2 cases; and films of splenic juice negative and cultures positive in 6 cases. In no less than 70 of the 76 cases the diagnosis could have been made by the examination of stained films of splenic juice obtained by puncture, but in 6 cases the films gave negative results when the cultures were positive.

Knowles' method of culturing the splenic juice for diagnosis consists of inoculating from 4 to 10 N.N.N. tubes with material obtained by puncture of the spleen, and examining the tubes at weekly intervals for a period of four weeks, the tubes being kept at a temperature between 18 and 28° C., the best results being obtained if the tubes are kept between 20 and 22° C. Smears are made from the water of condensation in the tubes. Bacterial contamination of the tubes quickly destroys the parasites, hence the necessity of preparing several tubes in each case.

**Direct Examination of the Peripheral Blood.**—That the peripheral blood of patients suffering from kala-azar contains *Leishmania donovani* in numbers that make its demonstration possible by microscopical examination of stained blood films is now well established, and this method of diagnosis is a most valuable one if properly applied. The percentage of

positive results obtained with it has varied considerably in the hands of different observers but the method is one that should always be thoroughly tried before one resorts to splenic puncture. In the peripheral blood the parasites are generally found within the mononuclear leucocytes, but they may also occur within the polymorphonuclear cells and, rarely, within the eosinophiles and free in the blood plasma. Several blood films should be thoroughly examined, using a mechanical stage on the microscope and covering each film thoroughly before the result is recorded as negative.

The frequency with which *Leishmania donovani* may be demonstrated in the peripheral blood is variously stated. Patton (1912-1914), an ardent advocate of this method of diagnosis, states that he has never failed but once in finding the parasites in the blood in cases of kala-azar originating in Madras, but several blood films often have to be examined before the parasites can be demonstrated. Thus, in the examination of the peripheral blood of 84 cases of the disease in Madras, he found *Leishmania donovani* in the first blood film examined in 42 cases, in the second in 13 cases, in the third in 12 cases, in the fourth in 5 cases, in the fifth in 2 cases, in the sixth in 4 cases, and in the remaining 6 cases, a larger number of films had to be examined before the parasites were found.

Korke (1914) confirmed Patton's results in Madras and Canata (1913-1914) found *Leishmania donovani* in the peripheral blood of 15 of 16 cases in Italy, but from 20 to 30 blood films had to be examined in each case.

Other authorities, however, have not had so favorable an experience with this method of diagnosis. Mackie (1915) examined the peripheral blood of 245 cases of kala-azar in Assam and found the parasite in only 21 per cent. of the native Assamese, and in 64 per cent. of the tea-garden coolies. Knowles (1920) examined 73 cases in Assam and was able to find the parasites in the peripheral blood in only 33, or 45 per cent., 682 blood films being examined, or an average of over 9 films to each patient.

Thick blood films were tried in the hope that they would increase the chances of finding the parasites, but proved unsatisfactory, but Knowles found that the hypodermic administration of 1 c.c. B. P. 1-1000 liquor adrenalin half an hour before the peripheral blood is examined increased the number of *Leishmania* in the blood. Using this method Knowles obtained 12 positive results in 18 cases of infection which, two hours before the administration of the adrenalin, had given only one positive result. Before the administration of adrenalin 95 blood films had been examined, while after its administration the 12 positive results were obtained from the examination of 50 blood films. It would appear from these observations that the administration of adrenalin prior to the ex-



amination of the peripheral blood for *Leishmania donovani* is a valuable method of increasing the chances of finding the parasite.

Marshall (1912) found *Leishmania donovani* in the peripheral blood of 80 per cent. of the cases examined in the Sudan, but in many of the cases a large number of films had to be examined before the parasites were encountered.

It is evident that *Leishmania donovani* occurs in the peripheral blood of a large proportion of, if not all, patients suffering from kala-azar, but that it is often very difficult, and, in some instances, impossible, to demonstrate them in this fluid. The method of diagnosis by direct examination of the peripheral blood demands great patience and is most laborious, but, nevertheless, it should always be employed before resort is had to puncture of the spleen. If carefully and conscientiously performed direct examination of stained films of the peripheral blood will give positive results in the vast majority of infections with *Leishmania donovani*.

Various methods have been proposed for increasing the value of peripheral blood examinations, as the use of thick blood films, and centrifuging the blood and making smears from the leucocyte layer in the sediment in the tubes, but neither of these methods has proven successful, as the parasites take the stain very poorly in both the thick blood smears and after centrifugation. With the exception of the administration of adrenalin, already mentioned, none of the methods that have been proposed have been found of any value.

In making blood smears for the demonstration of *Leishmania donovani* it should be remembered that the parasites are mostly contained within the leucocytes, and that films containing many leucocytes are most liable to give positive results. Thus rather thick films are to be preferred, and the edges of the film and the tail of the smear should be first examined as the leucocytes are most numerous in such areas of the film.

*Time of Occurrence of Leishmania donovani in Peripheral Blood.* Patton found that the parasites were most numerous in the peripheral blood during the late stages of kala-azar and when dysenteric symptoms were present, while other authorities claim that they are most numerous during the early stages of the disease and during the recurrent attacks of diarrhoea and dysentery. Knowles (1920) has investigated this question very carefully in Assam and concludes that the presence of *Leishmania donovani* in the peripheral blood "is a purely fortuitous phenomenon" occurring whenever the endothelial cells or leucocytes containing the parasites get into the peripheral circulation. While the parasites may be more frequently found during fever, dysentery, and in the terminal stages of kala-azar, such cases may give negative results, and very mild infections may show parasites in the peripheral circulation. Knowles



states that *Leishmania donovani* "is present, in scanty numbers, as a rule, in probably all, or in the majority of all, cases, in the peripheral blood."

**Culture of the Peripheral Blood.**—In view of the scarcity of the parasites in the peripheral blood and the time-consuming method of their demonstration by direct examination of blood films, it was believed that the cultivation of the parasites by inoculating N.N.N. medium with peripheral blood might give better results. The technique of blood culture for *Leishmania donovani* is as follows:

The blood is collected from a vein in the forearm, the usual precautions regarding the sterility of the syringe and needle and the site of puncture being observed. The blood obtained is diluted with normal saline solution, from one-fourth to one-half c.c. of blood being added to 20 c.c. of the salt solution and the mixture allowed to stand for 12 to 24 hours. The precipitate is then used for inoculating tubes of the N.N.N. medium, which are then incubated at a temperature between 20 and 22° C. for a period of at least four weeks, being examined at weekly intervals. Owing to the small number of parasites in the peripheral blood they may not appear in the cultures until two weeks after inoculation.

The results of blood cultures have varied greatly in the hands of different observers and there is a considerable difference of opinion regarding their diagnostic value. Wenyon (1914) was one of the first to demonstrate that *Leishmania donovani* could be cultivated from the peripheral blood, and Cornwall and La Frenais (1916) obtained positive results in 5 kala-azar patients in India, and Knowles (1920) cultured 12 cases in Assam, but obtained only 1 positive result. The latter author states that culture of the peripheral blood gave very disappointing results in his hands. Archibald (1923) appears to regard the culture of the peripheral blood as more certain in its results than the direct examination of blood films, but this opinion is not shared by most workers. However, if the direct examination has proven negative, blood culture should always be tried before resorting to splenic puncture.

Young and Van Sant (1923) have recently reported the recovery of *Leishmania donovani* in over 90 per cent. of untreated cases of kala-azar by their method of cultivation from the peripheral blood (see Appendix), the flagellates appearing in the cultures about ten days after inoculation of the medium. This method should be tried before splenic puncture is performed if the ordinary methods of examination of the peripheral blood prove negative.

**Differential Blood Count in Diagnosis.**—Certain changes occur in the total and relative number of the leucocytes in infections with *Leishmania donovani* which have been thought to be of diagnostic value. Rogers (1918) has called attention to the increase in the number of large mononuclear leucocytes in kala-azar and regards this increase of great diag-

nostic value but, unfortunately, a similar increase occurs in these cells in malarial infection, and, as malaria generally occurs in the endemic regions of kala-azar, the increase in large mononuclears alone is of comparatively little help in arriving at a diagnosis.

Knowles (1920), as the result of his study of the changes in the blood in infections with *Leishmania donovani*, concludes that the following are characteristic and of diagnostic importance.

1. The occurrence of large mononuclear leucocytes containing vacuoles in their cytoplasm.
2. A total leucocyte count below 4,000 per c.cm. with
  - a. A polymorphonuclear percentage of less than 50 per cent.
  - b. A large mononuclear and transitional leucocyte count of 20 per cent. or more.
  - c. An increase in eosinophiles.
  - d. Little or no change in the relative number of basophiles, lymphocytes, or small mononuclears.

These changes in the blood are considered by Knowles to be diagnostic, and in view of his results a total and differential leucocyte count should be made in every suspected case and due weight be given the results before splenic puncture is resorted to in diagnosis.

#### **Animal Inoculation of the Peripheral Blood and Splenic Juice.—**

The inoculation of susceptible animals with the peripheral blood or splenic juice of suspected cases has been resorted to in diagnosis, but the results have not been very satisfactory. This is largely due to the fact that animals are not very susceptible to infection with *Leishmania donovani* and massive doses of the virus are generally necessary in order to produce infection.

**Examination of the Lymphatic Glands.**—Puncture of enlarged lymphatic glands and the examination of the gland juice so obtained for *Leishmania donovani* by making stained smears has been used in diagnosis but has not been found to give very satisfactory results. Cochran (1913) recommends the excision of a superficial gland and the examination of smears made from the cut surface of the sectioned gland. This method is an improvement over gland puncture but has given positive results in relatively few cases.

**Serum Reactions in Diagnosis.**—Several methods of diagnosis in *Leishmania* infection based upon serum reactions have been advocated, but up to the present time none of them have proven to be as valuable as either splenic puncture or the direct examination of the peripheral blood.

The addition of water to the blood serum of kala-azar patients results in a primary turbidity followed by a flocculent precipitate. Brahmachari (1917) was the first observer to record this phenomenon, and he obtained a positive result with this test in 20 consecutive cases of kala-azar and

negative results in other diseases. Ray (1921) also obtained positive results with this test. Brahmachari recommended the addition of two parts of water to the blood serum, while Ray obtained the best results by placing two drops of the blood serum in a small tube and adding 20 drops of distilled water. Wenyon (1922) believes "that this peculiarity of the blood in kala-azar is worthy of further investigation."

*The Aldehyde Test.* Several observers have applied the "Formol gel Test" for syphilis, devised by Gaté and Papacostas (1920), to the diagnosis of infection with *Leishmania donovani*, and Napier (1922) and Mills (1922) have recently reported favorable results with a slight modification of this test. Napier calls attention to the fact that the specificity of this test in *Leishmania donovani* infection is in no way dependent upon gel formation, as it is in syphilis, and for that reason he suggests that the name of the test, when used in the diagnosis of kala-azar, should be distinctive, and he proposes that the test be known as the "Aldehyde Test."

The technique of the test, as recommended by Napier, is as follows:

About 5 c.c. of blood is withdrawn from a vein of the patient's forearm with the usual precautions regarding sterility. The serum is allowed to separate and 1 c.c. of the clear serum is placed in a small test tube, one-half inch in diameter, and to this is added one drop of 30 per cent. formaldehyde in the form of commercial formalin. The tube is thoroughly shaken and placed in a test-tube rack at room temperature.

The results of the test depend upon the degree of opacity of the blood serum after adding the formalin and the time consumed in solidification. In cases of kala-azar that have not been treated the serum will immediately become viscid and will solidify, so that the tube can be inverted, within a moment or two, at the same time becoming whitish and opalescent. In from three to twenty minutes the serum will have become solid and opaque, resembling the white of a hard-boiled egg.

In malaria, leprosy, phthisis, and other diseases there is no immediate change after adding the formalin, but after half an hour or less the serum becomes jellified, but no degree of opacity is observed until several hours, and then the opacity is never complete as in the blood serum of kala-azar patients.

The reaction tends to disappear after treatment, but Napier is uncertain as to how valuable it may prove as an index of the recovery of the patient.

Napier tested 150 consecutive patients whose symptoms were suspicious of infection with *Leishmania donovani* and who had been spleen-punctured. The results of the aldehyde test and spleen puncture agreed in 147 of the 150 cases, or 98 per cent. In 91 cases in which *Leishmania*

*donovani* was found in material obtained by splenic puncture, 89 were positive with the aldehyde test.

Mills (1922) tested 11 cases by both the aldehyde test and microscopic examination of the splenic blood and obtained positive results with both tests in 5 cases and negative results with both tests in 6 cases.

From the limited data available it is evident that the aldehyde test may prove a most valuable diagnostic method. It is exceedingly simple in technique and in the hands of Napier and Mills appears to have given excellent results. The test deserves very careful consideration, for if the results are as good in the hands of others as they have been in those of the investigators mentioned, it will certainly replace splenic puncture in the diagnosis of infection with *Leishmania donovani*.

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## CHAPTER XI

### THE BLOOD AND TISSUE FLAGELLATES OF MAN (CONTINUED).

#### LEISHMANIA TROPICA. DIAGNOSIS OF INFECTION WITH LEISHMANIA TROPICA. DOUBTFUL SPECIES OF LEISHMANIA.

IN THIS chapter *Leishmania tropica*, the well-known parasite causing Oriental sore or tropical ulcer, and certain doubtful species of *Leishmania* will be considered.

#### Species II. LEISHMANIA TROPICA, Wright, 1903

Synonyms: *Helcosoma tropicum*, Wright, 1903. *Ovoplasma orientale*, Marzinowski and Bogrow, 1904. *Crithidia cunninghami*, Carter, 1909. *Herpetomonas tropica*, Patton, 1912. *Herpetomonas furunculosa* (Wright), Patton, 1922? *Sporozoon furunculolum*, Firth, 1891.

**History and Nomenclature.**—*Leishmania tropica* was first described accurately and associated as the etiological agent with Oriental sore by J. H. Wright, in 1903, who found the parasite in an Armenian girl under treatment in the Massachusetts General Hospital, in Boston. Although to Wright belongs the credit of first accurately describing *Leishmania tropica*, it is practically certain that the parasite was seen by several observers before Wright (1903) published his description. Cunningham (1884) saw and described bodies in Delhi boil cases which were almost certainly identical with *Leishmania tropica*, while Riehl (1886) described similar bodies in a case of Aleppo boil but considered them encapsulated micrococci. In 1891, Firth found bodies in Oriental sore which he considered to belong to the PROTOZOA and which he named *Sporozoon furunculolum*, classing them with the *Microsporidia*, and it is evident, from his description, that he was, in all probability, dealing with *Leishmania tropica*.

Wright's observations were soon confirmed by James (1905) in India and by other observers, and, in 1908, Nicolle and Sicre were successful in cultivating *Leishmania tropica* on the N.N.N. medium and found that it developed into a flagellate in cultures similar in morphology to the cultural forms of *Leishmania donovani*. Patton (1912) demonstrated that, like *Leishmania donovani*, this species developed into a flagellate in the alimentary tract of the bed-bug (*Cimex hemiptera*), and Wenyon (1910) reported the development of this parasite in mosquitoes of the genus *Stegomyia*, while in 1915 the Sergeants and their co-workers described the development of *Leishmania tropica* in the sand-fly (*Phlebotomus papatasi*).

The nomenclature of this species is still a matter of controversy, both

the present generic and specific names being called in question by some authorities. *Leishmania tropica* is undoubtedly a *Herpetomonad* and the generic name *Leishmania* is considered by Rogers, Patton, and others as incorrect, and these authorities believe that the generic name should be *Herpetomonas*. However, as already stated in the description of *Leishmania donovani*, the generic name *Leishmania* applied to these parasites has become so firmly fixed in the literature that it would appear to be a mistake to change it at the present time.

Owing to the description of the parasite observed by Firth, which was published several years before that of Wright, many authorities believe that the specific name *furunculorum*, given by Firth to a parasite which they believe to be identical with *Leishmania tropica*, is the correct specific name of the latter organism, and that the specific name *tropica* given by Wright should be abandoned. According to these authorities the correct name of the parasite is *Leishmania furunculosa*, Firth, 1891. There is no question, that, according to the law of priority, as applied by zoologists in nomenclature, this name is correct if the parasite described by Firth was really identical with that described by Wright, but there still remains some doubt as to their identity in the minds of some observers, and as the name *Leishmania tropica* has become firmly fixed in the literature it should, in the opinion of most observers, be retained for this species of *Leishmania*.

**Morphology.**—In order to study the morphology of *Leishmania tropica* it is necessary to use stained preparations, and for staining either the Wright or Leishman stain is generally employed. The Wright stain has given most excellent results in my hands and is employed in the same manner as already described in staining *Leishmania donovani*, and the staining reactions of the parasite are the same.

In man the morphology of *Leishmania tropica* differs markedly from the morphology of the organism as observed in cultures and, therefore, it is necessary to describe the morphology of the parasite as observed in the infected human host and that observed in cultures in which the flagellate form is developed.

**Morphology in Man.**—The general morphology of *Leishmania tropica*, as observed in smears made from the lesions in man, resembles that of the forms of *Leishmania donovani* occurring in man so closely that it is generally stated that the two parasites are indistinguishable morphologically. In my experience, however, *Leishmania tropica*, while possessing the same structure as *Leishmania donovani*, is distinctly larger and coarser in appearance and appears to stain more readily and more intensely than does the latter parasite. However, these differences are only noticeable when one compares the two organisms in well-stained

preparations and are not sufficient to enable even experts to differentiate them under ordinary conditions.

The parasites occur as oval, oat-shaped, or spherical cells, either intracellular or free, and measure from 2 to 5 microns in length by 1 to 3 microns in breadth for the oval forms and from 2 to 3 microns in diameter when rounded. Longer and more slender forms are rarely observed, lying free and corresponding to the so-called torpedo forms of *Leishmania donovani*.

The organism is surrounded by a dimly stained capsule and the cytoplasm contains a large nucleus, the macro- or trophonucleus, and a minute blepharoplast. With the Wright stain the trophonucleus stains a rose red or pink and the blepharoplast a deep red or almost black color. The trophonucleus is situated either near the centre of the parasite or to one side in contact with the capsule, and the blepharoplast is situated close to and opposite the nucleus. The trophonucleus is round or oval in shape and may measure as much as 1.5 microns in length, while the blepharoplast is rod-shaped, round, or oval, and when rod-shaped is situated at an angle with the nucleus. A rhizoplast, consisting of a pale pink stained thread, may be sometimes seen arising apparently from the blepharoplast, but this structure is seldom seen except in very well stained preparations. The cytoplasm stains a pale blue, except in overstained specimens, when it may be dark blue, almost obscuring the nucleus and blepharoplast. Degenerative forms are often present in material from the sores, especially if secondary infection with bacteria is present.

*Leishmania tropica* occurs within the tissue cells, the endothelial cells, the large mononuclear leucocytes, and, less often, within the polymorphonuclear leucocytes. In some preparations large numbers may be found lying apparently free in the material obtained from the lesion, but in every preparation numerous intracellular forms will be found if carefully searched for.

Dividing forms may sometimes be seen, in which the blepharoplast, nucleus, and body are undergoing simple fission. Apparently fission is inaugurated by the division of the blepharoplast, followed by the division of the nucleus, and finally of the entire body. Binary division is the form most often seen, but some authorities describe a process of multiple division. I have never seen any evidences of multiple division in the forms of *Leishmania tropica* occurring in man.

**Morphology of Forms in Cultures.**—In cultures on the N.N.N. medium *Leishmania tropica* develops into flagellate forms similar to those already described as occurring in cultures of *Leishmania donovani*. After two or three days in the culture the round and oval forms observed in man are found elongated and pear-shaped, with one end much more pointed than the other. The size varies greatly, small flagellate forms



being observed that measure only from 3 to 7 microns in length and very long forms which may measure as much as 25 microns in length. The fully developed flagellate averages 18 to 20 microns in length and 1.5 to 4 microns in breadth. The flagellum measures from 10 to as much as 35 microns in length, being longer than the body in most specimens. The flagellate forms of this species average considerably longer than the flagellate forms of *Leishmania donovani*.

The structure of the flagellate form is similar to that of *Leishmania donovani*. There is a large nucleus or tropho-nucleus situated centrally or toward the aflagellate end, and a blepharoplast, round or oval in shape, situated at the anterior extremity of the parasite. The blepharoplast may be rod-shaped or semi-lunar in shape, especially just before division. Arising apparently from the blepharoplast is a long slender flagellum which projects anteriorly and becomes free almost at once. Forms are seen having two flagella and these are dividing forms which are seldom seen in cultures of *Leishmania donovani*. In cultures in which division is proceeding rapidly rosette formations occur composed of many flagellates arranged with their flagella all pointing inward and apparently inextricably mixed together. These rosettes are more common, in my experience, in cultures of *Leishmania tropica* than in those of *donovani*, probably due to the more rapid multiplication of the organisms.

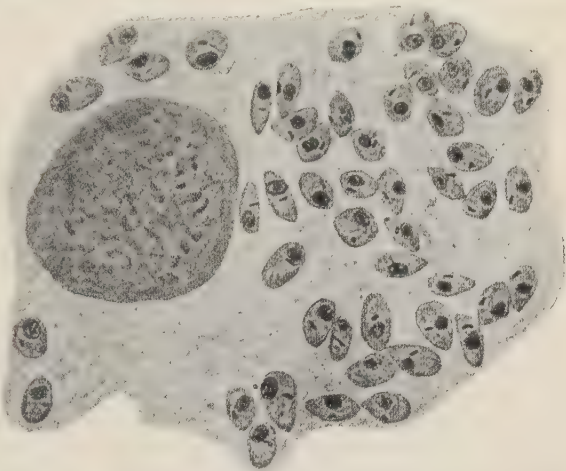


FIG. 59.—*Leishmania tropica*. A macrophage containing numerous *Leishmania tropica*. (After Patton.)

In old cultures the organisms lose their flagella and become rounded or oval in shape, and stain poorly. Such forms are interpreted by some observers as cystic forms and, if reinoculated into fresh culture media, will develop into normal flagellated forms.

In the cultures multiplication occurs by binary division, and, according to some authorities, by multiple division with the formation of several daughter flagellates. Sexual forms have been described, but that any sexual process occurs in cultures is very doubtful. I have never observed any phenomena that could be interpreted as conjugation or any forms of the parasite that resembled sexual forms.

**Habitat.**—*Leishmania tropica* is a parasite of the skin of man, being found in the tissue and endothelial cells of the area involved in the lesion. The parasites are also found within the large mononuclear leucocytes and in the polymorphonuclear cells in the blood that is mixed with the material obtained from scrapings of the ulcer, and lying free in the serum exuding from the ulcerated areas. Before ulceration *Leishmania tropica* is found at the margins and in the floor of the nodule, but after ulceration occurs it is most numerous in the tissue and endothelial cells at the margin of the ulcers. It may also be found, in small

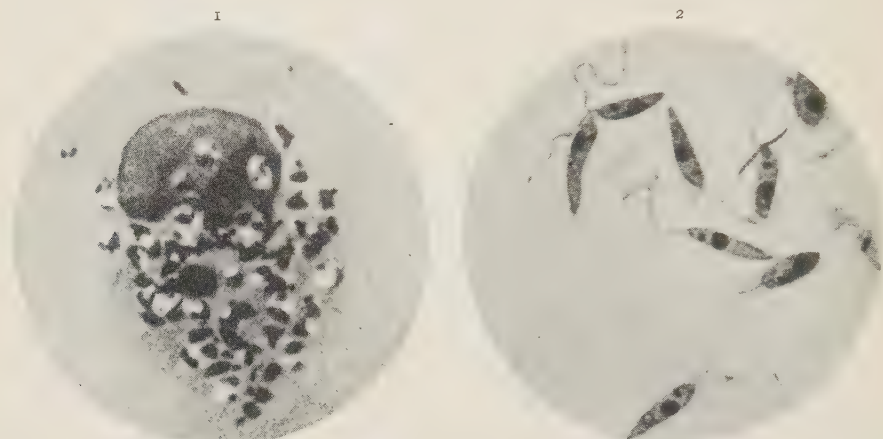


FIG. 60.—*Leishmania tropica*. (Photomicrographs. Army Medical School Collection.) Wright's stain. 1. *Leishmania tropica* in smear from a tropical ulcer.  $\times 1,800$ . 2. Flagellated forms of *Leishmania tropica* from a culture.  $\times 1,800$ .

numbers, in the peripheral blood obtained near the lesion, and it is possible, by culturing the blood, to obtain the parasite when it cannot be demonstrated in the blood by direct examination. In healing ulcers, or those that have existed for a long time, the parasites may be very small in number and difficult to find, and they are generally absent from the exudate if it is purulent or a secondary infection of the ulcer has occurred.

**Species Occurring in Lower Animals.**—Neligan (1913) demonstrated that *Leishmania* occurred in dogs in Teheran suffering from chronic ulcers of the head, the parasites being found in these ulcers, and Gachet (1915) confirmed his observations, finding ulcers in 15 of 21 dogs examined and recovering leishmania from the ulcers. Yakimoff and Schokhor (1914) found the same parasite in dogs in Tashkent, and, as it is impossible to distinguish these leishmania from *Leishmania tropica*, it is believed that natural infection in the dog may occur with this parasite.

There is no well-authenticated record of *Leishmania tropica* occurring in nature in any other animal, although the camel has been believed by some to act as a natural reservoir of the infection. The gecko and other

lizards have also been held by some authorities to be naturally infected and to act as reservoirs of infection for man, but without adequate proof.

The question of the occurrence of *Leishmania tropica* in insects will be discussed under the transmission of the infection, but it may be stated that the flagellate stage of this organism has been reported as occurring in mosquitoes, sand-flies, and the bed-bug.

**Cultivation.**—Nicolle and Sicre (1908) were the first to successfully cultivate *Leishmania tropica*, and for this purpose they used the N.N.N. medium as for culturing *Leishmania donovani*. As the parasites do not develop in cultures that are contaminated by bacteria it is essential that great care be used in securing material for this purpose. The skin over the nodule should be sterilized with iodine, after which a sharp glass pipette may be inserted into the nodule and the bloody fluid so obtained placed in the water of condensation in tubes of the N.N.N. medium, or the material may be obtained with a small glass syringe having a very sharp needle of large-sized bore. If the lesion is ulcerated the material should be obtained from the margin of the ulcer. Several tubes should be inoculated at the same time and kept at 22° C., if possible, but a variation in temperature between 20 and 25° C. is allowable. Development occurs within three or four days if the material inoculated is rich in parasites, but it may not occur until a period of three weeks or more if the parasites in the material inoculated are few in number.

In cultures *Leishmania tropica* develops into a flagellate resembling the flagellate forms of *Leishmania donovani*, and the morphology of these forms has already been described.

Archibald (1923) states that the flagellate forms of *Leishmania tropica* differ from those of *Leishmania donovani* in that the young cultures of the former contain numerous parasites having two flagella and that the flagellum of *Leishmania tropica* in cultures is longer than that of *Leishmania donovani*. Cultures of *Leishmania tropica* are less sensitive to variations in temperature and to contamination with microorganisms than are those of *Leishmania donovani*, according to Archibald, and he states that the Sudan strain of *Leishmania tropica* grows well in blood agar prepared with defibrinated sheep's blood, while *Leishmania donovani* in the Sudan will not develop on this medium.

Kligler (1924) has succeeded in cultivating *Leishmania tropica* in a medium modified from that used by Noguchi in the cultivation of *Leptospira icteroides*. The medium consists of a 1 per cent. agar mixed with 90 parts of normal salt solution to which, just before use, fresh sterile rabbit blood is added. The medium is then placed in tubes, each tube containing from 5 to 10 c.c., inoculated, sealed with liquid paraffin, and incubated at 25° C. (See Appendix.)

In these cultures flagellates begin to develop in from three to four days, although, if the material inoculated contained few organisms, the flagellates may not develop for a week or more. If the material is contaminated with staphylococci no development will occur in the cultures. Fresh cultures show many flagellates, but as the cultures grow older the flagellates gradually disappear and the round or oval forms seen in the tissues take their place. Kligler found that the original cultures remained alive for about eight weeks and that transplants lived for three months or even longer. He found that *Leishmania tropica* was very sensitive to changes in the reaction of the medium and that the best growth occurred when the reaction was pH 7.2, while no growth occurred at pH 7.8 or pH 6.6. The range of growth was between pH 7. and pH 7.6. Kligler's method of cultivation appears to be a valuable one and should be tried in the cultivation of *Leishmania donovani* also.

**Life-history.**—The entire life-cycle of *Leishmania tropica* is unknown. In man the parasite lives within the tissue and endothelial cells and multiplies by simple binary division, the blepharoplast first dividing, followed by the division of the nucleus and the body of the parasite. Some observers have described appearances which they have interpreted as schizogony, but there is no real evidence showing that multiple division occurs in the tissue or endothelial cells.

In cultures the flagellate forms divided by simple binary longitudinal division, in the same manner as in man, the division occurring first in the blepharoplast. Multiple division may occur in cultures, according to many observers, and the organisms may remain alive in cultures for weeks and subcultures may be carried along for years without losing their virulence. In the cultures the flagellates are actively motile, swimming about with the flagellum directed anteriorly.

In certain insects, as the sand-fly and the bed-bug, *Leishmania tropica* develops into flagellates similar to those occurring in cultures, and multiplication occurs in the same manner. In the bed-bug Adie (1921) and Patton (1921) claim that the parasite penetrates the cells of the intestine, and there undergoes schizogony, but their observations in this regard are considered doubtful by many investigators.

**Geographical Distribution.**—Although *Leishmania tropica* occurs in many regions in which *Leishmania donovani* also occurs, it is a peculiar fact that, in such regions, the two parasites do not occur together, but are more or less localized. Thus in India, *Leishmania tropica* occurs in the western portion, while *Leishmania donovani* occurs in the eastern districts. The parasite also occurs in regions where *Leishmania donovani* has never been found, as in Persia. There does not appear to be any connection between the two infections produced by these parasites so far as geographi-



cal distribution is concerned. However, both parasites occur in certain localities, as in the countries bordering upon the Mediterranean.

In Europe *Leishmania tropica* occurs in Italy, Sicily, Crete, and Spain. Rivaut (1920) has reported a case in France, contracted in the Pyrenees.

In Asia infections with this parasite occur in India in the northwest provinces, in the valley of the Indus, and in Cambay. The occurrence of these infections at Delhi gave rise to the name "Delhi boil," as applied to the lesions. In Syria, Aleppo is an endemic centre, hence the name Aleppo boil. In Asia Minor, Palestine, the Caucasus, and Transcaucasus, are well-known endemic centres of the infection. Infections are also common in Mesopotamia and in Turkestan. Cases have been reported from Asiatic Russia, but infections with *Leishmania tropica* have not been found in China, although infections with *Leishmania donovani* occur in certain endemic regions in that country.

In Africa infection with *Leishmania tropica* occurs in Tunis, Algeria, Morocco, Tripoli, Egypt, Abyssinia, Nigeria, the French Congo, the Tehad and Zinder districts, and the Sudan. The best-known endemic centres are Gafsa, in Tunisia; Biskra, in Algeria; and southern Morocco.

In America there occurs a form of cutaneous leishmaniasis known as espundia, or forest yaws, which is caused by a species of *Leishmania* very closely related to, if not identical with, *Leishmania tropica*. As the clinical condition produced by the American strain is quite distinct from that caused by *Leishmania tropica* it is considered by many excellent authorities that the parasite is a distinct species of *Leishmania*. For this reason the geographical distribution of this cutaneous leishmaniasis will not, at this time, be considered, but will be discussed in the portion of this chapter treating of *Leishmania braziliensis*.

**Incidence of Infection.**—In the endemic centres the incidence of infection with *Leishmania tropica* is very high, especially among infants and young children. Archibald (1923) states that in Aleppo it is very rare for a native to reach the age of 17 years without acquiring the infection, and this is also true of the population of other endemic centres. Epidemics of Oriental sore have been reported by several observers.

In Cambay, Patton (1922) states the liability to infection with *Leishmania tropica* is so great that "it is only necessary to spend a night there during the cold weather, and six months later a small papule appears on some exposed surface of the body" which becomes an Oriental sore, and from which the parasite can be recovered. Laveran (1917) states that, at Biskra, the smallest abrasion upon the skin is apt to become infected and develop into a typical sore, with *Leishmania tropica* present in the tissues and secretions. From these statements it is evident that infection with this parasite in the endemic centres is exceedingly common, and that few escape who remain there for any length of time.

**Method of Transmission.**—The method of transmission of *Leishmania tropica* from man to man is still unknown, although certain facts have been ascertained concerning it that point almost conclusively to insect transmission.

It is generally admitted that mere contact with patients suffering from Oriental sore does not lead to infection in most instances, but cases of infection by direct contact are reported. *Leishmania tropica* may be experimentally inoculated into healthy individuals with resulting infection, and the auto-inoculability of the parasite is well known. Thus Patton (1922) inoculated a man directly from a sore, due to *Leishmania tropica*, on his own person, the inoculation proving successful, and this man reinoculated himself from scratching and developed several sores on other parts of his body. From such experiments it is obvious that direct transmission by contact is possible, but it is the consensus of opinion that it seldom, if ever, occurs in nature.

**Insect Transmission.**—The fact that *Leishmania tropica*, like *Leishmania donovani*, develops a flagellated stage in cultures which must be kept at a temperature between 18 and 29° C., indicates that a part of its life-cycle is passed in a cold-blooded vertebrate or an invertebrate. The discharges from the sores or ulcers caused by *Leishmania tropica* are often rich in the parasites, and insects may thus easily become infected. The further fact that this parasite develops into a flagellate in certain insects points quite conclusively to insects being the transmitting agents, and various insects have been incriminated, including flies, mosquitoes, sand-flies, lice, fleas, and the bed-bug. At the present time sand-flies and the bed-bug appear to be the most probable transmitting agents, although it cannot be said that either has been actually proven to transmit the infection.

*Flies.* Various species of flies, especially the house-flies, have been thought by some observers to transmit *Leishmania tropica*. These insects feed upon the discharges from the ulcers produced by the parasite and upon the ulcerated tissue, and in this way must ingest numerous parasites, as well as soil their bodies and legs with material containing them. Whether such flies can transmit the infection to man is still a matter of controversy, but there would appear to be no reason why, if such an infected fly were crushed upon the skin where there was an abrasion, infection might not occur.

Blanc and Caminopetros (1921) allowed flies to feed upon sores rich in *Leishmania tropica*, and five hours later the heads and legs of the insects were crushed in liquid from the N.N.N. medium, and inoculated into the skin of the arm of healthy individuals, but no sores developed even after five months' observation. Patton (1922) has shown that *Leishmania tropica* never develops into a flagellate in the intestine of the fly, and his

experiments in endeavoring to infect clean cuts on his own person through the agency of supposedly infected flies were all unsuccessful.

Wenyon (1922) has suggested that it is possible that flies may ingest *Leishmania tropica* from the lesion and pass them within a few moments in their fæces upon abrasions on the skin of healthy individuals, and that in this way infection may occur. However, at the present time, there is no evidence of sufficient value to prove that *Leishmania tropica* is transmitted from man to man by house-flies, but more work remains to be done before the possibility of this method of transmission is eliminated.

*Mosquitoes.* Wenyon (1911) has described the development of *Leishmania tropica* in the alimentary tract of the mosquito *Aedes ægypti* (*Stegomyia fasciata*) at Bagdad, and claimed that it developed into a flagellate in the intestine of this insect, but Patton (1922) states that he has no doubt that the flagellate described by Wenyon was a natural *Herpetomonad* of the mosquito. It is thought at the present time that the transmission of *Leishmania tropica* by mosquitoes is very improbable, and the evidence supporting such a method of transmission is very slight.

*Sand-flies.* Flies belonging to the genus *Phlebotomus*, or sand-flies, as they are commonly called, have been suggested as the transmitting agents of *Leishmania tropica* by Pressat (1905), Wenyon (1911), the Sergents, Lemaire, and Senevet (1915), Patton (1919), and Laveran and Franchini (1920), and at the present time this theory appears to be more favorably regarded than any other, although it cannot be considered as proven experimentally.

In 1911, Wenyon observed flagellates in sand-flies at Aleppo, and the Sergents, Lemaire, and Senevet (1915) endeavored to produce infection in man, monkeys, and mice by the bites of *Phlebotomus minutus*, but were unsuccessful. In 1919, Patton found flagellates in *Phlebotomus papatasi* and *Phlebotomus minutus* in Macedonia, and Laveran and Franchini (1920) found the flagellates in *Phlebotomus papatasi* in France, obtained cultures on N.N.N. medium and claim to have produced a general infection in guinea-pigs and mice, and a local skin infection resembling Oriental sore in dogs. Confirmatory of Laveran and Franchini's experiments are those of the brothers Sargent, Parrot, Donatien, and Benguet (1921). These observers collected 94 sand-flies in the military hospital at Biskra, where Oriental sore is very prevalent, and sent them to Algiers, where the infection is unknown. Here the flies were macerated in normal salt solution and the mixture placed upon a scarified area on the arm of a healthy man. The scarification healed promptly, but two months and twenty-four days later a papular sore appeared upon the scarified area, which, upon examination, showed numerous *Leishmania tropica*.

The experiments mentioned would appear to prove that the sand-fly



can transmit a parasite that produces lesions like those produced by *Leishmania tropica*.

The question of the source of infection of the sand-fly with *Leishmania tropica*, otherwise than by biting infected man, has received a great deal of study since the Sergeants, Lemaire, and Senevet (1915) suggested that the gecko (*Tarentola mauritanica*) might act as a reservoir of infection for the fly, as sand-flies bite this lizard frequently, and a flagellate could be cultivated from the organs of a considerable proportion of the lizards. Chatton and Blanc (1918) claim to have successfully infected the gecko by intraperitoneal inoculations of *Leishmania tropica*, but Nicolle, Blanc, and Langeron (1920) determined that the flagellate found in the gecko naturally is morphologically different from *Leishmania tropica*, and were unable to infect either man or monkeys with the blood of naturally infected geckos or with cultures of the gecko flagellate. This work, as well as that of others, is quite conclusive that the gecko does not act as a reservoir of infection for sand-flies with *Leishmania tropica*.

Patton (1922) believes that in Mesopotamia the sand-fly is the transmitting agent of *Leishmania tropica*, stating, "In Mesopotamia, where Oriental sore is very common and widely distributed, the sand-fly seemed to me to be the only possible insect which could be the host of the parasite." He believes that *Leishmania tropica* in Mesopotamia is the vertebrate phase of the *Herpetomonad* of *Phlebotomus papatasi*, and that man is infected by crushing the infected sand-fly upon the skin when the insect is biting.

Much remains to be accomplished before the transmission of *Leishmania tropica* by the sand-fly can be accepted as proven, but the evidence available points very strongly to the conclusion that this insect is the real transmitting agent, and that the parasite passes a portion of its life-cycle within the alimentary tract of flies belonging to the genus *Phlebotomus*.

*Lice.* There is no evidence that lice act as transmitting agents of *Leishmania tropica*, and Patton (1922) states that all of his experiments in this direction gave negative results.

*Fleas.* Although the flea has been believed by some observers to transmit *Leishmania tropica*, there is no evidence that any species of flea can transmit the infection.

*Bugs.* The bed-bug (*Cimex hemiptera*) is believed by Patton to be the transmitting agent of *Leishmania tropica* in India. He has shown that, in the alimentary tract of this bug, the parasite becomes a flagellate, and Patton, La Frenais, and Rao (1921), from careful studies upon the development of the parasite in the bed-bug, have reached the following conclusions:

"When an adult bug is fed on cultures of *Herpetomonas tropica* the



flagellates pass down to the rectum, where they can be found in the living condition 24 hours after the feed.

"In microscopic preparations the parasites can be found in the alimentary tract of the bug as late as the nineteenth day after the feed.

"From microscopic examinations alone it would appear that the parasite disappears from the mid-gut of the bug, if it is not refed after the original feed of the culture.

"If a bug is refed again on clean human blood after a short interval, a large number of round growing flagellates appear in its mid-gut contents, and these by multiplying produce an intense infection."

The observers mentioned above have also determined that *Leishmania tropica* can live for 23 days in the alimentary canal of starved bed-bugs, for 34 days in the stomach of refed bugs, for 44 days in the hind-gut, and for 34 days in the rectum. In the refed nymph the parasite can live for 31 days in the mid-gut and in the hind-gut and rectum for 36 days. In the larval bug they found that *Leishmania tropica* could live in the mid-gut for at least nine days. These results were obtained by culturing portions of the alimentary tract of infected bugs upon the N.N.N. medium.

Patton (1921-1922) has recently found intracellular stages of development of *Leishmania tropica* in the cells of the mid-gut of the bed-bug similar to those described by Adie (1921) as occurring in the same situation in bugs infected with *Leishmania donovani*, but Cornwall and La Frenais (1922) have not been able to confirm Patton's results.

Patton (1922) believes that the infection of man is brought about by crushing the infected bed-bug upon the skin while the insect is biting, and instances two cases in which Oriental sore developed on the skin where infected bugs were crushed.

While Patton's theory of the transmission of *Leishmania tropica* by the bed-bug is attractive and plausible, and while there appears to be no doubt that the parasite develops into a flagellate in the alimentary tract of this bug, it lacks the conclusive evidence of actual experimental transmission of the infection to man or any of the lower animals by this insect.

**Transmission by Ingestion of Contaminated Food.**—It has been suggested that *Leishmania tropica* might be transmitted to man by means of contaminated food or drink. The only way in which food could be contaminated would be through the agency of insects, either directly, by the soiled feet or bodies of insects reaching food or drink, or indirectly, through the droppings of an infected insect, or through the agency of soiled hands of food handlers. As infection with this parasite is localized in the skin, the parasite, if taken in through the mouth, would have to pass through the wall of the stomach or intestine and, reaching the blood stream, be carried to the skin, where development must occur. Such a method of transmission is most improbable, and there is no reason to believe that the ingestion

of *Leishmania tropica* in food or drink would be followed by infection of the skin and the production of the characteristic lesion.

**Experimental Infection of Lower Animals.**—*Leishmania tropica* can be experimentally transmitted to several of the lower animals, especially to monkeys, dogs, and mice. Nicolle and Sicre (1908) were the first to demonstrate that the subcutaneous inoculation of monkeys with material from Oriental sores containing the parasite, or cultures containing the flagellated form, is followed by the appearance of typical lesions in these animals, and that *Leishmania tropica* can be recovered from the lesions. Nicolle and Manceaux (1910) repeated such experiments on dogs with successful results, and since these observations many observers have been successful in transmitting the parasite to monkeys and dogs and the etiological relationship of *Leishmania tropica* to Oriental sore has thus been proven. The incubation period in monkeys and dogs extends over several weeks, and the duration of the disease in these animals is from 12 to 18 months.

Gonder (1913) inoculated mice intraperitoneally and intravenously with cultures of *Leishmania tropica* and obtained a general infection with local lesions on the head, legs, and tail. Subinoculations from mouse to mouse resulted in infection and the production of similar lesions. Laveran confirmed Gonder's results in mice and obtained similar results from the inoculations of cultures of the parasite in guinea-pigs, gerbils, and rats. The lesions produced in all of these animals were positive for *Leishmania tropica*. Immunity is not conferred by the inoculations, as Nicolle and Laveran have shown that reinoculation after recovery from the initial infection is followed by infection. A partial immunity has been observed in monkeys and dogs by some observers, and most authorities state that in man one attack usually confers a lasting immunity.

**Relation to Disease.**—*Leishmania tropica* is a pathogenic parasite, causing in man a cutaneous lesion generally known as Oriental sore, but which is given a great number of local names connected with the endemic centres in which it has been observed. Thus, the condition is called Delhi boil, Biskra boil, Aleppo boil, frontier sore, and tropical sore. The lesion consists of an ulcerating or nonulcerating granuloma of the skin, usually occurring on some exposed surface of the body, especially upon the face, arms, hands, and legs. The incubation period is long, varying from two months to one year, although an incubation period as short as two weeks has sometimes been observed. In the endemic centres infants and young children are most frequently attacked, as most adults have had the infection in early life. There is no distinction as to sex, and individuals in good health are as liable to be infected as those who are debilitated. A seasonal prevalence of the infection is noted, as in India, where the infection is most

common in the colder months. The duration of the disease is from a few months to a year or more.

The growth of the parasites in the tissues of the skin leads to a hypertrophy of the stratum corneum with hypertrophy and proliferation of the papillæ. The diseased skin contains areas of infiltration with plasma cells, lymphoid cells, and large mononuclear leucocytes. Very large cells containing multitudes of the parasites may be present, and nests of cells, resembling those present in epitheliomata, are observed. Necrosis and ulceration occur and, owing to the exposed situation of the lesions, secondary infections with bacteria are very common. Unlike infection with *Leishmania donovani*, the changes in the blood are slight, anæmia being absent, and there is no marked increase in the mononuclear leucocytes. There may be a slight increase in the large mononuclears and the eosinophiles, but the changes are in no way diagnostic.

Multiple lesions are frequently encountered, as the infection is auto-inoculable.

The causative relation of *Leishmania tropica* is undoubted. The parasite is always found in the characteristic lesion, can be cultivated from the lesion, and the cultures reproduce in susceptible animals the typical lesion of the infection as observed in man. Many observers have inoculated themselves with this parasite, and have developed the lesion, and such experimental infections have been transmitted from man to man by inoculation and by rubbing material containing the parasite into abrasions on the skin.

**Prophylaxis.**—In the absence of exact knowledge as to the method of transmission it is obvious that prophylactic measures can only be empirical. From the evidence that has accumulated regarding the probable connection of insects with the transmission of *Leishmania tropica* it follows that protection of the body from the bites of insects, especially from sand-flies and bed-bugs, is an important prophylactic measure. In order to protect from sand-flies a netting having 22 holes to the linear inch is necessary. If bitten by insects the bites should be sterilized at once with iodine. Infected individuals should have their lesions covered to prevent the access of insects to them, as well as to prevent secondary infections and auto-inoculations.

While *Leishmania tropica* is probably seldom transmitted by direct contact, the danger of this method of transmission should be remembered, and individuals that are infected should be instructed concerning the possible danger of conveying the infection to others in this manner.

The rôle of the dog in the transmission of the infection is still undetermined, and it is the part of wisdom to avoid contact with them as much as is possible. In the endemic centres dogs have been found infected, and this fact renders their suppression in such districts advisable.

**Diagnosis of *Leishmania tropica*.**—The diagnosis of *Leishmania tropica* depends upon finding the parasite in the involved tissues or in material obtained from the nodules or ulcers produced by its growth in the tissues.

Smears may be prepared from material obtained by scraping the edges of the ulcerated areas after removing the crust or scab covering the ulcers. These smears should be stained with the Wright or Leishman stain in the same manner as already described in the discussion of the diagnosis of *Leishmania donovani*. (See Chapter X.) In such material the parasites are not numerous, as a rule, and often stain very poorly. If such is the case a sharp glass capillary pipette may be forced into the base of the ulcer, and the material so obtained stained and examined. Such material often contains numerous parasites when smears from the scrapings of ulcers are negative. The staining reactions of *Leishmania tropica* have already been described in the discussion of the morphology of the parasite.

When ulceration has not occurred material may be obtained for staining by aspiration of the nodule with a small glass syringe, or puncture of the nodule with a sharp capillary glass pipette, the material so obtained being smeared upon microscopic slides and stained.

It is sometimes impossible to demonstrate the presence of *Leishmania tropica* in material obtained from nodules or ulcers, and, when this is the case, resort should be had to cultures of such material upon the N.N.N. medium. Extreme care should be taken in the collection of material for cultures to avoid contamination with bacteria, for *Leishmania tropica* will not grow in cultures if contamination occurs. For this reason it is useless to culture scrapings of the ulcers and the material should be obtained from the tissue beneath the floor of the ulcer or its edges. Wenyon (1922) recommends that the skin at the margin of the ulcer be sterilized with iodine, and that the tissue beneath the floor of the ulcer be reached by running a fine glass pipette through a puncture made in the skin into the desired area. The material so obtained generally consists of a mixture of blood and cells, and should be inoculated into the water of condensation in tubes of N.N.N. medium, and the cultures kept as near a temperature of 22° C. as is possible. Several tubes should be inoculated, as often only one or two tubes of a dozen will show growth.

If numerous parasites are present in the material inoculated the flagellates may be found in the water of condensation in the tubes in from 48 to 72 hours, but if the parasites were few in number several weeks may elapse before the flagellates will be demonstrable in the cultures. The cultures should be examined every three or four days for a period of one month before they are discarded as negative.

Although *Leishmania tropica* has been reported as present in the periph-



eral blood obtained in the vicinity of the lesion, cultures of the peripheral blood have not given positive results, so far as I am aware, and are of no use in diagnosis.

In the vast majority of cases of infection with *Leishmania tropica* the parasites can be demonstrated in stained smears made from material obtained directly from the nodules or ulcers if care be used in the collection of the material and patience in the examination of the preparations. Several stained smears should be examined before this method of diagnosis is abandoned in favor of the cultural method.

The occurrence of small oval or round bodies, possessing a large nucleus and a minute blepharoplast, and lying within the tissue cells, endothelial cells, large mononuclear leucocytes, or polymorphonuclear leucocytes, is diagnostic of *Leishmania tropica* when the material examined is obtained from suspected lesions of the disease in man.

### DOUBTFUL SPECIES OF LEISHMANIA

There have been several species of the genus *Leishmania* described which are of doubtful status. As already stated, the supposed species, *Leishmania infantum*, long regarded as a valid species, is now held, by the best authorities, to be identical with *Leishmania donovani*. A species of *Leishmania* occurring in Mexico, Central and South America, and causing a cutaneous disease known under various local names, is held by some authorities to be a distinct species or variety of *Leishmania tropica*, while the strain of *Leishmania* observed in the Sudan was thought to be a distinct variety by Archibald and others. These organisms will be briefly described, but it is my personal belief that the American leishmania is not identical with *Leishmania tropica*, but that the Sudan strain is identical with *Leishmania donovani*.

### LEISHMANIA BRAZILIENSIS, Vianna, 1911

Synonyms: *Leishmania tropica*, Wright, 1903, variety *Americana*, Laveran and Nattan-Larrier, 1912.

**History and Nomenclature.**—Lindenberg (1909) and Carini and Paranhos (1909) found a parasite, belonging to the genus *Leishmania*, in ulcers occurring in man in Brazil, and their observations were confirmed by Nattan-Larrier, Touin, and Heckenroth (1909), and Carini (1911), who found leishmania in the lesions of the naso-pharyngeal mucous membrane associated with the ulcers, and by Escomel (1911) and Laveran (1912). Pedrosa and Da Silva (1910) were the first to demonstrate that this parasite could be cultivated upon the N.N.N. medium, and that in cultures it developed into a flagellate.

Vianna (1911) studied the leishmania described by the authors

mentioned, and considered it a new species, naming it *Leishmania braziliensis*. Escomel (1911) sent his preparations to Laveran to be identified, and Laveran identified the parasite as belonging to the *Leishmania*, but noted differences which he believed indicated that it was a variety of *Leishmania tropica*, and gave it the name *Leishmania tropica*, var. *Americana*.

The lesions produced by the American parasite were said to differ from those produced by *Leishmania tropica*, and this was held by those believing in the specific status of the parasite as proving its specific nature, but Castellani (1913) has reported a case of infection with *Leishmania tropica* in Ceylon presenting the same lesions as those observed in infections with the American parasite, and similar cases have been reported by Christopherson (1914) and Susa (1917) in the Sudan. In view of these findings, and the similarity of the American parasite morphologically, culturally, and experimentally to *Leishmania tropica*, it appeared very doubtful if *Leishmania braziliensis* could be regarded as a valid species, but the recent work of Noguchi (1924) upon the immune reactions of these two species indicates that they are distinct. He found that the serum of animals immunized to *Leishmania tropica* did not agglutinate cultures of *Leishmania braziliensis*, and *vice versa*.

**Morphology.**—The morphology of *Leishmania braziliensis* is like that of *Leishmania tropica* according to the majority of observers who have studied the parasite. In both man and cultures the morphology appears to be the same. Some observers call attention to the flattening of the nucleus, the deeper staining reactions, and the longer flagellum of *Leishmania braziliensis* as distinguishing and specific features of its morphology, but these differences are far from sufficient upon which to base a species, and all of them have been observed in undoubted examples of *Leishmania tropica*. What has been said of the morphology of the latter species is equally true of *Leishmania braziliensis*.

**Habitat.**—This leishmania lives in the tissue cells, endothelial cells, and large mononuclear cells in the involved portions of the skin of man. In addition, the parasite may be found in the same cells in the affected mucous membrane of the nose, mouth, and pharynx. It may also be found in the polymorphonuclear leucocytes, but is phagocyted by these cells and apparently cannot multiply within them.

There is some evidence that *Leishmania braziliensis* may live and multiply within the alimentary tract of certain flies of the genus *Forcipomyia*.

**Species Occurring in Lower Animals.**—There is only a single instance reported of *Leishmania* occurring naturally in any of the lower animals in South America. Pedrosa (1913) reported the occurrence of a leish-

mania indistinguishable in morphology from *Leishmania braziliensis* in an ulcer in the nose of a dog examined in an endemic centre of the infection in Brazil. If this parasite is identical with *Leishmania tropica* it is probable that further research will show that the infection is more or less prevalent in dogs in the endemic centres.

Townsend (1915) has reported finding a flagellate in the alimentary tract of a fly belonging to the genus *Forcipomyia* which he believes to be identical with *Leishmania braziliensis*.

**Cultivation.**—*Leishmania braziliensis* can be cultivated upon the N.N.N. medium without difficulty and develops into a flagellate similar in morphology to the flagellate stage of *Leishmania donovani* and *Leishmania tropica* in cultures. Pedrosa and Da Silva (1910) were the first to find the flagellate stage of this parasite in cultures, and their observations have been confirmed by numerous investigators. Bonne (1919) found that cultures made from the peripheral blood gave negative results. The cultures must be kept as near 22° C. as possible for development to occur, and are made in the same manner as described for *Leishmania tropica*.

**Life-history.**—The entire life-history of this organism is unknown, but in man it lives in the tissue cells, the endothelial cells, and in large mononuclear leucocytes of the skin and multiplies by simple binary division in the same manner as *Leishmania tropica*. Nothing is known concerning the life-history of the parasite in insects, if an insect is the invertebrate host, which is probable.

**Geographical Distribution.**—*Leishmania braziliensis* is widely distributed in endemic centres in Mexico, Central, and the northern half of South America. In Brazil the infection is wide-spread, and has been reported by numerous observers. In Peru, the Harvard Commission, headed by Strong (1913), studied infections produced by this parasite, and Iturbe (1917) observed cases in Venezuela. In the Guianas the infection is known as "Forest Yaws" and has been studied by Nattan-Larrier, Touin, and Heckenroth (1909); Flu (1911); and Bonne (1918). The parasite has been found in Colombia by Tejera (1920); in Paraguay by Migone (1913); in Yucatan by Seidlin (1912); in Bolivia by Escomel (1917); in Mexico by Inchaustegui (1918); and in Panama by Darling (1911). Castellani has observed a case in Ceylon, and cases have also been reported from the Sudan.

The infection is localized in well-known endemic centres, and a large proportion of the population of such centres suffers from the infection.

**Incidence of Infection.**—There is little data on record giving in detail the incidence of infection with this parasite in the endemic districts. It is most prevalent in forest regions, and least so in large towns and cities. Strong (1913) and his co-workers found the infection very prevalent in a

village in the mountains in Peru, and states that a large proportion of the inhabitants showed infection or lesions of past infection. Inchaustegui (1918) found 50 per cent. of chicle gum collectors in certain regions in Mexico to be infected, and da Silveira (1920) observed 15,000 cases at San Paulo, Brazil, in a period of five years. In certain regions the infection is rare, as in Panama, where only a comparatively few cases have been reported, and in certain localities in all countries where the infection is endemic.

**Method of Transmission.**—The method of transmission of *Leishmania braziliensis* is unknown, but the little evidence that is available points to its transmission by some biting insect. As in the case of *Leishmania tropica*, biting flies have been suspected, and Townsend (1915) claims to have secured an infection in a guinea-pig by inoculations with crushed flies of the genus *Forcipomyia*, belonging to the CHIRONOMIDÆ. Sand-flies are considered as the transmitting agents by some authorities, and *Phlebotomus lutzi*, the common species found in the endemic areas, has been incriminated, although there is no experimental evidence that this insect transmits the parasite, beyond the fact that a few isolated instances have been reported of the development of the initial lesion at the site of the bite of this insect. Ticks have also been thought to transmit the infection.

As the disease is directly inoculable it is possible that direct contact may result in infection, but this method of transmission is not believed to be the usual one.

**Experimental Infection of Lower Animals.**—*Leishmania braziliensis* has been experimentally transmitted to the baboon, monkeys, dogs and cats, guinea-pigs, and, with difficulty, to rats, mice, and rabbits.

**Relation to Disease.**—*Leishmania braziliensis* is a pathogenic parasite, causing a form of cutaneous and muco-cutaneous leishmaniasis in Mexico, Central and South America, characterized by lesions of the skin similar to those produced by *Leishmania tropica* and, in addition, by the production of nodules, ulcerations, and necrosis of the mucous membranes of the nose, mouth, and pharynx, leading to great deformity and mutilation. The latter lesions are undoubtedly due to the combined action of *Leishmania braziliensis* and secondary bacterial invaders of the infected tissues. The parasite is always present in the lesions, and produces similar lesions in susceptible animals.

The pathological lesions in the skin consist of a marked infiltration of the involved area with lymphocytes, large mononuclear leucocytes, endothelial cells, and macrophages, all of which may contain numerous parasites. The epidermis is thickened and edematous, and epithelial nests may be noted in the sections. Eventually the tissue undergoes necrosis, forming an ulcer, and parasites may be demonstrated in the tissue forming the



edges and floor of the ulcer, lying in the connective tissue cells, the endothelial cells, and in the large mononuclear cells.

In the lesions of the mucous membranes the epithelial layer is destroyed, and the mucous membrane is infiltrated and œdematous, the infiltrated areas presenting large numbers of plasma cells, endothelial cells, and large mononuclear leucocytes, with congestion and blocking of the capillary vessels and consequent production of minute necrotic areas. The parasite may be found within large mononuclear and endothelial cells in the superficial layers of the involved membrane and at the edges of the ulcerations.

The period of incubation is not known. The initial lesion is sometimes followed years afterward by the muco-cutaneous lesions, and the infection may last for many years, and often terminates fatally through secondary infections of various kinds. However, only a certain proportion of the infected individuals develop lesions of the mucous membrane, for da Silveira (1920) found involvement of the mucous membranes of the nose, mouth, and pharynx in only 20 per cent. of the 15,000 cases he studied at San Paulo, Brazil.

**Prophylaxis.**—The prophylaxis of infection with *Leishmania braziliensis* should follow the same line as already described for *Leishmania tropica*. There is no evidence that insanitary conditions predispose to this infection, for the sores may be contracted while travelling through virgin forests, as proven many times by the experience of explorers and workers on railroad construction in South America. It would appear that the most important prophylactic measure is the protection of the person from the bites of insects.

**Diagnosis.**—The diagnosis of the infection depends upon finding the parasite in the lesions, and the same methods are employed as already described in the discussion of the diagnosis of *Leishmania tropica*.

#### LEISHMANIA DONOVANI, var. ARCHIBALDI, Castellani and Chalmers, 1920

This is a very doubtful variety of *Leishmania donovani*, discovered in the Sudan, by Archibald, in 1913. Castellani and Chalmers accept it as a distinct variety, but Archibald (1923) states that neither morphologically nor culturally does it differ from *Leishmania donovani*, and evidently regards it as identical with the latter species, an opinion held by most authorities who have studied the Sudan parasite. The reasons given for considering the parasite as a distinct variety, or subspecies, are that it presents a coccal stage in its life-history, and appears to be transmitted through infected food rather than by the bite of an insect. As neither of these reasons is supported by scientific evidence it is evident that this so-called variety of *Leishmania donovani* should no longer be recognized,

and that the Sudan parasite should be regarded as identical with *Leishmania donovani*.

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## CHAPTER XII

THE COCCIDIA OF MAN. *ISOSPORA HOMINIS*. *EIMERIA WENYONI*.  
*EIMERIA OXYSPORA*. *EIMERIA SNIJDERSI*. THE COCCIDIUM OF  
THE LIVER. THE DIAGNOSIS OF THE COCCIDIA OF MAN.

The COCCIDIA are a well-defined class of the SPOROZOA, and are considered to be rare parasites of man. However, the work of English investigators during the World War has demonstrated that a considerable percentage of soldiers serving in certain localities became infected with coccidia, and a careful investigation of the population of such regions will undoubtedly result in showing that coccidial infection of man is not so rare as is now believed by most authorities.

Most of the species of coccidia occurring in man are parasites of the intestinal canal, where a portion of their life-cycle is passed within the epithelial cells of the mucous membrane. The COCCIDIA are true tissue parasites, and although no symptoms are apparently produced by their presence in man, the fact that they invade and ultimately cause the destruction of the host cells is proof that, however minute the lesion may be that they produce, they must be regarded, in the strict sense of the term, pathogenic parasites of man.

**Life-history.**—The life-history of the COCCIDIA has been worked out largely upon species parasitic in the lower animals. Little was known regarding the complicated life-history of the COCCIDIA until the researches of Schaudinn and Siedlecki (1897) were published, and even today the entire life-cycle of none of the described species has been actually demonstrated.

The following description of the life-cycle of typical coccidia is based upon the descriptions and researches of Schaudinn and Siedlecki (1897) and Schaudinn (1900).

The COCCIDIA, like the plasmodia of malaria, have an asexual and a sexual stage of development, in both of which their morphology differs greatly.

In the *asexual* stage of development the coccidium is a small oval organism living within a tissue cell of its host, and in the earliest stage of development in this situation is called a *schizont*. The *schizont* gradually enlarges within the host cell until it nearly fills it, the cell degenerating as growth advances until all that remains of it is a narrow film of cytoplasm, forming a delicate membrane surrounding the parasite. When fully grown the *schizont* reproduces by multiple division, the nucleus dividing into several parts, followed by the division of the cytoplasm. This process of division of the *schizont* is called sporulation by some authors, and the



entire asexual life-cycle is known as *schizogony*. The young coccidia produced by the division of the *schizont* are called *merozoites* and are motile, fusiform bodies, which, liberated from the parent *schizont* by the rupture of the cell membrane, bore their way into other tissue cells and repeat the

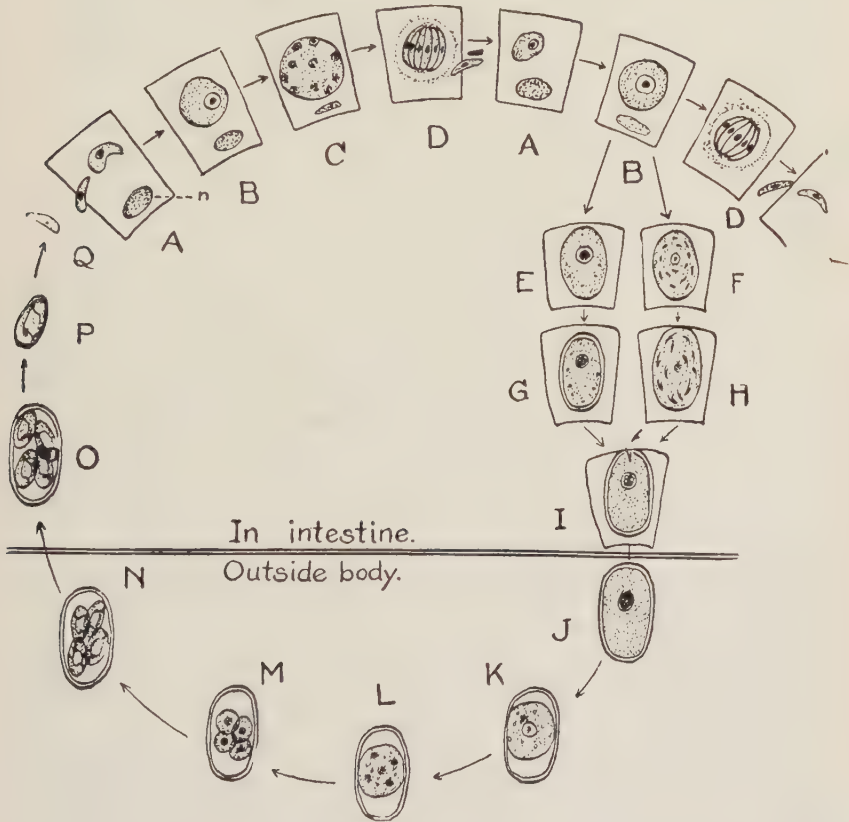


FIG. 61.—Life-cycle of a typical coccidium, *Eimeria avium*. A. Infection of epithelial cells of intestine by sporozoites ingested with food and water. B. Growth inside cell. C and D. Sporulation and formation of young spores. E and G. Formation of female gametes. F and H. Formation of male gametes. I. Fertilization. J. Fully developed oocyst as passed in faeces. K, L and M. Formation of four sporocysts. N. Complete development of sporocysts, each containing four sporozoites. O. Same, ingested by susceptible animal. P. Sporocyst liberated from oocyst in alimentary canal. Q. Liberated sporozoite ready to enter epithelial cell, as shown in A. (After Chandler.)

process of *schizogony*, becoming *schizonts* as soon as they reach the host cell.

*Schizogony* may be repeated for many generations, but eventually it ceases and the *merozoites* become differentiated into male and female forms, and the sexual cycle, or *sporogony*, is initiated.

In the sexual cycle of development, as stated, the *merozoites* are differentiated into male and female forms, the male form being known as the *microgametocyte*, and the female as the *macrogametocyte*. The nucleus of

the *microgametocyte* undergoes division and several flagellate forms are thus produced which are called *microgametes*. At the same time certain maturation phenomena take place in the nucleus of the *macrogametocyte* which prepares it for fertilization, and it is then known as the *macrogamete*. The *microgametes* are liberated from the parent *microgametocyte*, all that is left of the latter being a residual mass of granular cytoplasm, and swimming actively about, come in contact with the *macrogamete*, and one of the *microgametes* penetrates the *macrogamete* through a tiny opening in the limiting membrane and fertilizes it. The process of fertilization may occur either within or outside of the host cell, but generally outside the tissue cell.

The fertilized *macrogamete* is called a *zygote* or *oöcyst*. As a rule the *oöcyst* is extracellular, and nuclear division occurs within it, resulting in the formation of numerous small bodies known as *sporoblasts*, and a residual mass of granular material representing that portion of the *oöcyst* which is not used in the formation of the *sporoblasts*. Each *sporoblast* secretes a cystic membrane about itself and becomes a *sporocyst*, and the *sporoblasts* are now known as *spores*. The nucleus of the *spore* divides into several daughter nuclei, followed by the division of the cytoplasm, thus producing a number of sickle-shaped vermicular forms which are called *sporozoites*, a small portion of the cytoplasm of each *spore* remaining as a residual body.

Under favorable conditions the *spores* containing the *sporozoites* rupture and liberate the latter. If this occurs in the natural host of the parasite the *sporozoites* penetrate the host cell and become *schizonts*, and thus the asexual cycle of development, or *schizogony*, is renewed. The *sporozoites* are the only forms of the coccidia that are able to cause infection in a new host.

**Classification.**—The classification of the COCCIDIA is based upon the number of *sporozoites* present in the *spores* and the number of spores within the *oöcyst*. Until Dobell's revision of the *Coccidia*, in 1919, the classification of these organisms was in a chaotic condition, but his laborious and painstaking study of these parasites has resulted in a classification that is based upon a firm and scientific foundation.

The first coccidium discovered was found in the liver of a rabbit by Hake, in 1839, but was not recognized by him as a parasite, and it was not until nearly 50 years later that the coccidia were generally accepted as protozoan organisms, largely owing to the researches of Rivolta (1878), Leuckart (1879), and Balbiani (1884).

The coccidia of the intestine of the dog and cat have been extensively studied by numerous investigators. Virchow (1860), Grassi (1879), Stiles (1891–1892), Swellengrebel (1914), Wenyon (1915), and Dobell (1919) have all contributed valuable data regarding the nature, mor-

phology, and life-history of various species of the *Coccidia*, or have reported instances of infection of man by these parasites. Wenyon (1915-1915 a-1915 b) described two species which he found in the fæces of man, and in 1919, Dobell described a third species which he found in human fæces.

According to Dobell (1919) the coccidia of man all belong to two genera, *Isospora* and *Eimeria*, identified as follows:

Genus I. *Isospora*, Aimé, Schneider, 1881. ( : *Diplospora*, Labbé, 1893.) Oöcyst contains two tetrazoic spores.

Genus II. *Eimeria*, Aimé Schneider, 1875. ( : *Coccidium*, Leuckart, 1879.) Oöcyst contains four dizoic spores.

In the genus *Isospora* there is one species that has been found in the human intestine and in the genus *Eimeria* three species parasitic in the human intestine, and one very rare species parasitic in the human liver.

### Genus I. ISOSPORA, Aimé Schneider, 1881.

Synonym: *Diplospora*, Labbé, 1893.

This genus was founded by Aimé Schneider (1881) to include an organism which he found in a slug. While his description of this parasite is unsatisfactory, it is sufficiently clear to enable one to be sure that the organism was a coccidium. The genus *Isospora* was accepted by Labbé (1893) but he interpreted the genus as applied to forms having two polyzoic spores and established a new genus, *Diplospora*, for forms having two tetrazoic spores. Labbé's classification is accepted by Leger (1911), but the majority of students of these parasites, including Schaudinn (1900), Laveran and Mesnil (1902), and Dobell (1919), regard Labbé's genus *Diplospora* as a synonym of *Isospora*, and this interpretation is followed in this work.

The only species of the genus *Isospora* occurring in man, so far as known, is *Isospora hominis*, a parasite of the small intestine.

### Species I. ISOSPORA HOMINIS (Rivolta, 1878), Dobell, 1919.

Synonyms: *Cytospermium hominis*, Rivolta, 1878. *Coccidium perforans*, Leuckart, 1879. *Coccidium bigeminum* (Stiles), var. *Hominis*, Railliet and Lucet, 1891. *Coccidium hominis* (Rivolta), Labbé, 1896. *Coccidium bigeminum* (Stiles), Blanchard, 1896. *Eimeria stiedæ* (Lindemann), Lühe, 1906. *Isospora bigemina* (Stiles), Doflein, 1911. *Isospora*, Wenyon, 1915.

**History and Nomenclature.**—*Isospora hominis* was probably first seen by Kjellberg, in 1860, as recorded by Virchow (1860), in the villi of the small intestine of man. Eimer (1870) confirmed its occurrence in this situation, and Rivolta (1878) named the organism *Cytospermium hominis*. The oöcysts were first found in the fæces by Railliet and Lucet (1890), but the first detailed and accurate account of the oöcysts and spores was furnished by Wenyon (1915-1915 a). Dobell (1919) in his

revision of the *Coccidia*, described the parasite in detail, and collected and reviewed the records of cases of infection with it that have been published. He also named this species *Isospora hominis*.

**Morphology.**—Only the oöcysts of this species have been seen, the schizogonic stage of development being unknown, as well as the sexual development.

The oöcysts are found in human stools in an unsegmented condition, but develop spores after removal from the body. They are elongate oval



FIG. 62.—*Isospora hominis*.  $\times 2,000$ . (After Dobell.) 1. Oöcyst, with unsegmented protoplasm, as usually passes in stools. 2. Fully developed oöcyst, containing two spores, each spore containing four sporozoites.

in shape and measure from 25 to 33 microns in length by 12.5 to 16 microns in breadth, one end of the oöcyst is narrowed, and presents a neck-like appearance. Some of the oöcysts are long and slender, while others are shorter and close to spheroidal in shape.

Wenyon (1923) believes that the two forms of oöcysts mentioned belong to two distinct species, the smaller being those of *Isospora hominis* and the larger those of a new species, which he has named *Isospora belli*.

The wall of the oöcyst consists of two layers, and is smooth, thin and colorless. The inner layer is membranous, while the outer is hard and very resistant to the passage of fluids. A very small micropyle may be noted at the narrow end of the cyst, but is generally invisible.

When passed in the fæces the contents of the oöcysts are in an unsegmented condition in most instances, appearing as a spherical mass filled with bright refractile granules. Rarely oöcysts are observed in the freshly passed fæces containing two sporoblasts. The nucleus in the oöcyst can be distinguished as a large clear area somewhere within the granular mass.

The further development of the oöcysts occurs outside the body and, according to Dobell (1919) and Dobell and O'Connor (1921), in the following manner: The nucleus in the mass of granular cytoplasm first divides into two portions, followed by the division of the entire mass within the cyst into two daughter cells or sporoblasts. The two sporoblasts become oval in shape and secrete a cyst wall, thus becoming a sporocyst, eventually becoming spores which have a double wall and measure from 12 to 14 microns in length by 7 to 9 microns in breadth.

The further development of the spores within the oöcyst consists in



the division of the single nucleus within the spore by two successive divisions into four nuclei, each of which becomes the nucleus of a sporozoite, four sporozoites being thus formed within each spore. A mass of cytoplasm is left after this division, which is known as the sporocystic residue. The sporozoites are long, slender, somewhat crescent-shaped bodies, having a single nucleus at one end, and lie clumped together within the spore in a granular mass, which is the sporocystic residue.

In this species there is no oöcystic residue, all of the cytoplasm of the oöcyst having been used up in the formation of the sporocysts. Wenyon and O'Connor (1817) described an atypical development of some of the oöcysts of this species in which a single sporocyst is formed in which eight sporozoites are finally developed.

After the development of the sporozoites no further development of the parasite occurs until the oöcysts are swallowed by man when the sporozoites are liberated in the intestine, become motile, and, penetrating the epithelial cells of the mucous membrane, initiate the asexual cycle of development, or schizogony.

**Habitat.**—*Isospora hominis* is a parasite of the small intestine of man. As noted, only the oöcysts of this species are known, but from what is known of the life-cycle of closely related species in the lower animals it is believed that schizogony and certain stages of the sexual life-cycle are completed in the epithelial cells of the mucous membrane of the small intestine.

**Species Occurring in Lower Animals.**—A very closely related species, *Isospora bigemina*, occurs in the intestinal villi of dogs, cats, and polecats. This species is commonly known as *Coccidium bigeminum*, and has been confused with the human species until recently.

**Cultivation.**—Has not been cultivated.

**Life-history.**—The probable life-history of *Isospora hominis* has already been described, but it has never actually been demonstrated. The ripe oöcysts after being swallowed by man are believed to liberate the sporozoites in the small intestine, and these, penetrating the cells of the villi, undergo schizogony and eventually sporogony, with the consequent development of the oöcysts which are later found in the stools.

**Geographical Distribution.**—Cases of infection with *Isospora hominis* have been reported from Egypt, Gallipoli, Salonika, the Balkans, Macedonia, Mesopotamia, and South Africa. It has also been reported from France, Tripoli, Senegal, and the United States.

**Incidence of Infection.**—According to Dobell (1919), about 70 cases of infection with this parasite have been reported, and since he wrote a few more have been recorded. Most of the cases occurred among soldiers invalided during the World War to England from Gallipoli, Salonika, Mesopotamia, and Egypt. Haughwout (1921) has traced 12 cases of infection in the United States.

Wenyon (1916) found 15 infections with this parasite during the stool examinations of 556 soldiers. Woodcock (1915) and Woodcock and Penfold (1916) have described 10 cases, and Dobell (1916) an additional case. Roche (1917) found 15 infections in a total of 893 patients he examined, suffering from dysentery or diarrhœa acquired at Salonika, and Savage and Young (1917) found 6 cases of infection among 1,088 soldiers that they examined. Cragg (1917) reported 4 infections among 613 dysentery and diarrhœa patients, and Boney, Crossman, and Boulenger found 7 infections among 890 soldier patients. Porter (1918) reports two cases of infection in South Africa with *Isospora hominis*, one in a Hottentot and one in a European who was born there and had never left the country.

From these data it would appear that infection with this parasite is not so rare as has been supposed, and that in some localities it is as commonly observed as some of the intestinal flagellates. Further observations will probably show that it is frequently encountered in the countries named above, and that it may be present in other localities. It is my belief that the oöcysts have been frequently overlooked or mistaken for vegetable cells or other objects by those who have never seen them, and that a more careful study of the subject will show that *Isospora hominis* is not, by any means, a rare parasite in certain localities.

**Method of Transmission.**—The method of transmission of the infection is unknown, but it is, in all probability, through food or drink contaminated by the oöcysts of the parasite. Porter (1918) claims that the oöcysts of this species pass unchanged through the intestinal canal of the common house-fly and green-bottle flies, and these insects may be a possible means of transmission of the parasite to man.

**Experimental Infection of Lower Animals.**—Dobell (1919) states that all attempts to infect animals with *Isospora hominis* have failed, and this species has never been found in any of the lower animals. Porter (1918) states that it produces diarrhœal symptoms in kittens, but Wenyon and O'Connor (1917) fed kittens and mice with mature spores without producing an infection.

**Relation to Disease.**—There is no evidence that *Isospora hominis* is a pathogenic parasite so far as the production of symptoms is concerned. As all *Coccidia* invade and live upon the tissue cells of their host, ultimately causing the destruction of these cells, it follows that in every case of infection microscopic lesions due to their presence and growth must be present in the infected tissues. These lesions, though they may not cause symptoms, are proof of the essential pathogenic nature of the *Coccidia* and *Isospora hominis* is probably not an exception to the rule. Therefore, while this species apparently does not cause symptoms in man, it should not be forgotten that it destroys tissue, and in some instances, if the para-

site was present in great numbers, symptoms present might be due to it. In most of the cases in which it has been found in the fæces there was present diarrhœa and dysentery, but either one of the dysentery bacilli or *Endamæba histolytica* was also found and could account for the symptoms, so that it is impossible to be sure in any of the cases reported that the symptoms were due to *Isospora hominis*. The parasite has also been found in perfectly healthy individuals, but only in small numbers and at irregular intervals. At present it cannot be claimed that *Isospora hominis* is a pathogenic parasite if that term be confined to a parasite that causes symptoms of disease in its host.

Most infections with this organism are transient, the oöcyst soon disappearing from the stools, although infections may persist for some time. It may be that some of the persistent cases have been due to reinfection. Dobell (1919) states that infections with this species are more persistent than infections with the other coccidia of man.

**Prophylaxis.**—So far as known prophylaxis depends upon personal hygiene and the protection of food and drink from contamination by the oöcysts of the parasite.

## Genus II. EIMERIA, Aimé Schneider, 1875.

Synonym: *Coccidium*, Leuckart, 1879.

The genus *Eimeria* was founded by Aimé Schneider, in 1875, and following the studies of Schaudinn (1900) and Stiles (1902), become generally accepted. Species belonging to this genus are characterized by having oöcysts containing four spores, each of which contains two sporozoites. There are three species belonging to this genus that are parasitic in the intestine of man, i.e., *Eimeria wenyoni*, *Eimeria oxyspora*, and *Eimeria snijdersi*, and one poorly studied and unnamed species parasitic in the human liver.

### Species I. EIMERIA WENYONI, Dobell, 1919.

Synonyms: *Eimeria* (*Coccidium*), Wenyon, 1915. *Eimeria*, sp. Dobell, 1917. *Eimeria*, sp. Dobell and Stevenson, 1917. *Eimeria*, Roche, 1917.

**History and Nomenclature.**—This species was discovered by Wenyon, in 1915, in the fæces of a soldier invalided to England from Gallipoli. Roche (1917) found the same parasite in the fæces of three patients examined at Salonika. Dobell (1919), in his revision of the *Coccidia* of man, named the parasite *Eimeria wenyoni*. In its morphology it closely resembles *Eimeria falciformis*, a common coccidium of the mouse.

**Morphology.**—The only form of *Eimeria wenyoni* that has been studied is the oöcyst, as this is the only form occurring in the fæces, and the description which follows is compiled from those of Wenyon (1915) and Dobell (1919).

The oöcysts of this species as seen in the faeces are segmented and fully developed, unlike those of *Isospora hominis*, which are passed in an unsegmented condition. They are spherical in shape and measure about 20 microns in diameter. The outer surface of the oöcyst is rough, while the inner surface is smooth and covered with a delicate membrane. The cyst contains four oval spores, measuring 10 by 7 microns, with roughened outer surfaces. There is no residual mass of granules in the oöcyst. Each spore contains two falciform sporozoites, one end being more rounded than

the other. The rounded end contains the nucleus, a spherical or oval area which appears hyaline. The blunt ends of the sporozoites lie at opposite ends of the spore and between the sporozoites, or near them, there are one or two refractile granular masses, the sporocystic residue.



FIG. 63.—*Eimeria wenyoni*. An oöcyst, containing four fully developed spores.  $\times 2,000$ . (After Dobell.)

**Habitat.**—This species is a parasite of the small intestine of man, but the only stage in the life-cycle that has been studied is the oöcyst, which is found in the faeces. In all probability the asexual and most of the sexual stages of development are passed within the epithelial cells of the intestinal mucous membrane.

**Species Occurring in Lower Animals.**—*Eimeria wenyoni* has never been found in any of the lower animals, but a species that resembles it very closely, *Eimeria falciformis*, is a common coccidium of the mouse. Other species somewhat resembling it are *Eimeria avium*, a parasite of game birds and poultry; *Eimeria stiedæ*, a common coccidium of rabbits; and *Eimeria zürni*, which occurs in cattle. These species have often been confused with the human species, but there is no doubt that they are all distinct from *Eimeria wenyoni*.

Davis and Reich (1924) have described two new species of *Eimeria*, one occurring in sheep and the other in hogs, but do not name the organisms.

**Cultivation.**—*Eimeria wenyoni* has not been cultivated.

**Life-history.**—The life-history of this species is unknown, as only the fully developed oöcysts have been discovered. It is presumed that it has the same asexual and sexual cycles of development common to all the *Coccidia*, and that it develops within the epithelial cells of the small intestine of man.

**Geographical Distribution.**—The only instances of infection with *Eimeria wenyoni* that are recorded occurred in Gallipoli and Salonika.

**Incidence of Infection.**—It is safe to say that *Eimeria wenyoni* is a



very rare parasite of man. Wenyon (1915) saw it only once in 556 patients from Gallipoli whose faeces he examined, and Roche (1917) found only three infections in 893 men examined at Salonika.

**Method of Transmission.**—How the infection is transmitted is unknown, but undoubtedly contaminated food and drink are responsible. The oöcysts are the infective agents.

**Experimental Infection of Lower Animals.**—So far as I know there is no record of the successful transmission of this parasite to any of the lower animals.

**Relation to Disease.**—There is no evidence that *Eimeria wenyoni* is a pathogenic parasite in so far as the production of symptoms is concerned.

**Prophylaxis.**—The prevention of the infection depends upon personal hygiene and the protection of food and drink from contamination by the oöcysts.

## Species II. EIMERIA OXYSPORA, Dobell, 1919.

Synonym: *Eimeria oxyphila*, Mesnil, 1919.

**History and Nomenclature.**—*Eimeria oxyspora* was described by Dobell, in 1919. He found this species in the faeces of a single patient suffering from a coincident infection with *Endamæba histolytica*. Its morphology differed from that of any described species, and Dobell named it *Eimeria oxyspora*.

**Morphology.**—The following description of the morphology of this species is compiled from that of Dobell (1919).

The only stage in the life-cycle of *Eimeria oxyspora* that is known is the fully developed oöcyst, which is found in the faeces of infected man. The oöcyst is spherical in shape and measures about 36 microns in diameter. The wall of the cyst is slightly yellowish in color and is composed of two layers, the outer, of composite character and incrustated with particles from the faeces, and the inner, thicker and uniform in appearance.

Within each oöcyst there are four long, sharply pointed, whetstone-shaped spores, measuring from 30 to 32 microns long by about 7.5 microns in breadth. The spores are covered by a wall (sporocyst) composed of a tough uniform inner coat and a thinner and slightly uneven outer coat. Besides the spores, the oöcysts contain a small, more or less loosely arranged collection of refractile granules, the oöcystic residue.

Each spore contains two long, slender sporozoites which almost fill the spore, the anterior end of each sporozoite being pointed and wrapped around the posterior end of the other sporozoite. The posterior end of the sporozoites is rounded and contains a nucleus. Between the nucleus and the posterior end there are two or three bright, crystal-like bodies arranged longitudinally. These bodies, according to Dobell (1919), are not found in the sporozoites of any other species of coccidium. Between the nucleus

and the anterior end of the sporozoite there are a few small, slightly refractile granules within the cytoplasm.

The posterior, nucleated end of the sporozoites lies at opposite poles of the spore. There is a small sporocystic residue within the spore, consisting of a few refractile granules distributed near the centre of the spore.

**Habitat.**—This species is a parasite of the intestine of man, probably the small intestine.

**Species Occurring in Lower Animals.**—*Eimeria oxyspora* has not been found in any of the lower animals, nor has any species been found in the lower animals that resemble it closely in morphology.

**Cultivation.**—This species has not been cultivated.

**Geographical Distribution.**—Unknown. The one individual recorded by Dobell as infected with this parasite had been in South Africa, Ceylon, and India. Broughton-Alcock and Thompson (1922) have recorded one case of infection in London.

**Life-history.**—Only the oöcysts are known, so the life-history of this species has not been demonstrated. It is presumably like that of other of the *Coccidia*.

**Incidence of Infection.**—*Eimeria oxyspora* is apparently a very rare parasite of man. Dobell found it in only one case, and then the oöcysts were in small numbers, and appeared irregularly in the faeces.

**Method of Transmission.**—Unknown, but probably through the contamination of food and drink by the oöcysts of the parasite.

**Experimental Infection of Lower Animals.**—There is no record of the experimental transmission of *Eimeria oxyspora* to any of the lower animals.

**Relation to Disease.**—Dobell (1919) found this species of coccidium in a patient suffering from dysentery due to *Endamæba histolytica*, and a coincident infection with *Anchylostoma*. Regarding the question of pathogenicity of *Eimeria oxyspora* he says (page 190):

“The *Eimeria* infection was so small that, even if it had caused any symptoms, it would hardly have been possible to distinguish these from

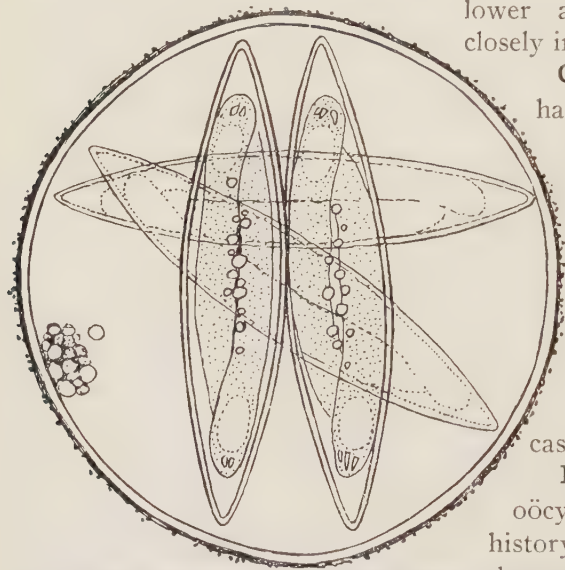


FIG. 64.—*Eimeria oxyspora*. An oocyst, containing four fully developed spores.  $\times 2,000$ . (After Dobell.)

the effects produced by the accompanying parasites. At present there is no indication that *Eimeria oxyspora* is pathogenic, though since it is, like all coccidia, a tissue parasite, it is doubtless capable of doing damage to its human host."

### Species III. EIMERIA SNIJDERSI, Dobell, 1921.

*Eimeria snijdersi* was discovered by Snijders, in 1921, in the fæces of a single individual in Sumatra, and named by Dobell in the same year.

The only stage of development that is known is the fully developed oöcyst, which was found in the fæces. These are spherical in shape and measure 40 to 48 microns in diameter, the average diameter being about 45 microns. The wall of the oöcyst consists of two definite layers, and the oöcyst contains four dizoic spores and a small oöcystic residue composed of scattered granules.

The spores are spindle-shaped and measure from 22 to 24 microns in length by 7.5 microns in breadth. The walls are double, and within each spore there are two long worm-like sporozoites. A sporocystic residue is also present in each spore, consisting of one or two very refractile globules. One end of the sporozoite is more rounded than the other, which is pointed, and in the rounded end a nucleus is sometimes visible, but no crystalline bodies as in the sporozoites of *Eimeria oxyspora*. The sporozoites of this species are shorter and more plump than are those of *Eimeria oxyspora*, and the oöcysts are considerably larger, measuring on the average 45 microns in length as compared with 36 microns, the average length of the oöcysts of *Eimeria oxyspora*.

There is nothing known of the life-history of *Eimeria snijdersi* or its habitat in the body beyond the fact that the fully developed oöcysts were found in the fæces. It is believed that its life-cycle occurs within the epithelial cells of the mucous membrane of the small intestine.

### Species IV. EIMERIA ? SP. (The coccidium of the liver of man.)

Synonyms: The synonyms of this species are many, but the following are the most important: *Coccidium oviforme*, Leuckart, 1879. *Coccidium cunniculi* (Rivolta), Blanchard, 1896. *Eimeria stiedæ* (Lindemann), Lühe, 1906. *Eimeria stiedæ* (Lindemann), Doflein, 1911. *Coccidium cunniculi* (Rivolta), Brumpt, 1913. *Eimeria stiedæ* (Lindemann), Jollos, 1913.

There have been five cases reported of infection of the liver of man by a coccidium. These cases were reported by Gubler (1858), Dressler (Leuckart, 1863), Perls and Sattler (Leuckart, 1879), Perls and von Sömmering (Leuckart, 1879), and Silcock (1890). It is impossible from any of the descriptions given of the parasite found to properly identify this coccidium, but it is certain that it is not identical with *Eimeria stiedæ*, the coccidium of the liver of the rabbit, although many authors state that the two are identical.

The little that is known regarding the human species proves that the oöcysts are ovoid in shape and measure about 20 microns in length, while the oöcysts of *Eimeria stiedæ* measure from 30 to 37 microns in length. Aside from the shape and size of the oöcysts nothing is known of the morphology of this species, as no one has described the contents of the oöcyst, the only observer who appears to have seen the segmented oöcyst being Silcock, but his description does not give the number of spores or sporozoites. Dobell (1919) has called attention to the fact that the drawings of the oöcyst of this species by Dressler do not in the least resemble the oöcyst of *Eimeria stiedæ*, and he regards it as remarkable that so many observers, for so long a period of time, have regarded the two species as identical, when such an opinion had to be based upon the drawings and descriptions that have been published.

The lesions produced by this species in man are similar to those produced by *Eimeria stiedæ* in the rabbit. In those patients in which it has been found there was enlargement of the liver, digestive disturbances and fever. The parasites were found in the liver, in the biliary ducts, and in the spleen. In the liver and the biliary ducts they were found within the epithelial cells.

Until this coccidium is studied more thoroughly than it has been it is unwise to be dogmatic regarding its exact specific position, and as Dobell (1919) well says: "It is clear that further observations alone can solve the problems connected with the human parasite, and the naming of it may therefore be left to some future investigator who is fortunate enough to find it once more."

**The Diagnosis of the Coccidia of Man.**—The diagnosis of the coccidia occurring in the intestine of man rests upon the recognition of their oöcysts in the fæces, as none of them present any diagnostic symptoms due to their presence in the intestine.

The fæces should be examined as soon after passage as possible, and in unstained preparations, as the oöcysts do not stain well, and all of the details of the structure of the cyst can be easily made out in the unstained fæces. A small portion of the fæces is mixed with a little normal salt solution or distilled water, placed upon a microscopic slide, and gently flattened out with a cover-glass. The oöcysts may be picked up with the low-power dry objective (16 mm.) but the structures within the oöcyst are best studied with the 4 mm. dry and 1.9 mm. oil immersion objectives. Careful attention should be paid to illumination, as the oöcysts and their contents are practically colorless, and only perfect illumination will reveal their minute structure with accuracy. Undoubtedly the oöcysts have been many times overlooked in the fæces because of the use of too much light in making the examination, and it should always be remembered that as



little light as is possible to secure a good image should be used in studying these structures.

The morphology of the oöcysts of all of the intestinal coccidia is so striking that it should be easy to recognize them, but unless the details of their morphology are remembered it might easily be possible to confuse them with certain vegetable cells that sometimes are found in the fæces or with other bodies of extraneous origin. However, it is very probable that the mistake of interpreting vegetable cells or other bodies as coccidia is much more apt to occur, and this has been done repeatedly by well-trained observers. Yeast cells and blastomycetes have been mistaken for coccidia many times, and this fact should be remembered when cases of infection with intestinal coccidia are reported.

The cysts of *Isospora hominis*, *Eimeria wenyoni*, *Eimeria oxyspora*, and *Eimeria snijdersi* can be identified if one follows carefully the differential features given by Dobell and repeated in this work. In arriving at a diagnosis of any body believed to be a coccidium belonging to any of the species mentioned, attention should be paid to the size of the oöcyst, the number of spores within the oöcyst, and the number of sporozoites within the spores. When passed in the fæces the spores of *Eimeria wenyoni*, *Eimeria oxyspora*, and *Eimeria snijdersi* are segmented and the sporozoites developed within the spores, but it should be remembered that in the case of *Isospora hominis* the oöcysts, when passed in the fæces, are not segmented, and it is this particular oöcyst that may be mistaken for certain vegetable cells. If the fæces are allowed to stand for some time segmentation occurs, and then the morphology of the oöcyst is much more typical. However, no difficulty should be encountered in the diagnosis of the oöcysts of any of the coccidia if careful attention is paid to details of morphology that are to be expected if the object one is examining is suspected of being the oöcyst of one of the intestinal coccidia.

The diagnosis of the species of *Eimeria* that has been reported as parasitic in the liver of man depends upon the finding of the oöcysts in the contents of the bile-ducts or in the liver tissue. Where symptoms have been present that were suspicious of coccidial infection of the liver a careful examination should always be made for the oöcysts, and the fæces should also be examined, for if the infection is in the bile-ducts there would appear to be no reason why the oöcysts should not be found in the fæces under certain conditions.

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## CHAPTER XIII

THE MALARIA PLASMODIA. CLASSIFICATION AND NOMENCLATURE.  
THE GENUS PLASMODIUM. HISTORY AND NOMENCLATURE. LIFE-  
CYCLE IN MAN AND MOSQUITO. SPECIES OF PLASMODIA.  
CULTIVATION OF PLASMODIA. GENERAL MORPHOLOGY  
OF PLASMODIA. METHOD OF TRANSMISSION.  
PLASMODIA IN LOWER ANIMALS.

The malaria plasmodia are protozoan parasites belonging to the SPOROZOA, order HÆMOSPORIDIA, family PLASMODIDÆ, and genus *Plasmodia*.

The HÆMOSPORIDIA include a large number of protozoan parasites living in the blood cells of their vertebrate host during a portion of their life-cycle. All of them pass through an asexual cycle of existence in a vertebrate host in which reproduction occurs by schizogony, and a sexual cycle in an invertebrate host, in which reproduction occurs by sporogony. So far as is definitely known resistant spores are not formed in either the asexual or sexual cycle, as in the COCCIDIA.

The HÆMOSPORIDIA may be conveniently divided into three great families, the PLASMODIDÆ, the HÆMOGREGARINIDÆ, and the PIROPLASMODIDÆ. Of these three families the only one containing parasites of interest in human pathology is the PLASMODIDÆ. This family contains four well-defined genera, *Plasmodium*, *Hæmoproteus*, *Proteosoma*, and *Leucocytozoon*, and all of the hæmosporidia of man are found in the genus *Plasmodium*.

The family PLASMODIDÆ is composed of protozoan parasites which pass through an asexual cycle of existence within the red blood corpuscles of a vertebrate host, or, in the case of the genus *Leucocytozoon*, in the leucocytes of the vertebrate host. During this cycle of development there is produced, through the destruction of the host cell, characteristic pigment known as melanin, or hæmozoin. In the genus *Leucocytozoon* pigment formation is not observed. The sexual cycle of existence is passed in some species of mosquito except in the case of the parasites belonging to the genus *Leucocytozoon*. In this family there are included parasites of the red blood corpuscles of man, dogs, antelopes, monkeys, squirrels, bats, birds, and reptiles, and of the leucocytes of birds.

As stated, the genus *Plasmodium* is the only genus included in the family PLASMODIDÆ that contains parasites of interest in human pathology. This genus contains only organisms that pass the asexual cycle of existence in the red blood cells of the vertebrate host and the sexual

cycle in some species of mosquito. Besides the species of plasmodia that live in the red blood corpuscles of man, similar species occur in some of the lower animals, as will be noted later.

Genus. PLASMODIUM, Marchiafava and Celli, 1885.

Synonyms: *Oscillaria*, Laveran, 1881. *Hæmatomonas*, Osler, 1887. *Hæmatophylum*, Metchnikoff, 1887. *Hæmamæba*, Grassi and Feletti, 1889. *Laverania*, Grassi and Feletti, 1889. *Cytamæba*, Danilewski, 1890. *Protosoma*, Labbé, 1894. *Hæmosporidium*, Lewkowicz, 1897. *Cytosporon*, Wasielewski, 1901.

**History and Nomenclature.**—The genus *Plasmodium* was established, in 1885, to include the protozoan parasites discovered in the red blood corpuscles of patients suffering from malaria by Laveran, a French army surgeon, in 1880. The history of this discovery is so well known that it will not be reviewed here, except to state that Laveran first found the parasites in cases of malaria observed at Constantine, Algeria. His descriptions and interpretations of the etiological relationship of the parasites to malaria were so bitterly opposed by zoologists and medical scientists, even after confirmation by well-known workers, that it was not until 1890, ten years after his discovery, that it was generally accepted that the bodies described by him were the cause of malarial fevers in man. Laveran established the genus *Oscillaria* for the malaria plasmodia, but as this name had already been used in designating a plant it had to be abandoned, and, in 1885, Marchiafava and Celli suggested the generic name *Plasmodium* for the malaria parasites, a name which is now generally accepted as the true generic name.

In 1889, Grassi and Feletti divided the genus *Plasmodium* into two genera, *Hæmamæba* and *Laverania*. In the first they included all malaria plasmodia of spherical shape, and in the second, plasmodia of crescentic shape. They believed the crescentic *gametes* of the æstivo-autumnal plasmodia to be a distinct genus of plasmodium, and called this genus *Laverania*. Some recent writers accept the genus *Laverania*, including in it the æstivo-autumnal plasmodia, but it is not believed that such a position is to the best interests of zoological nomenclature so far as the malaria plasmodia are concerned. While there is no doubt that the crescentic shape of the *gametes* of the æstivo-autumnal plasmodia is sufficient upon which to base a genus, the fact that the generic name *Plasmodium* has become firmly fixed in our nomenclature for all the species of plasmodia, and that only confusion will be caused by using another generic name for certain species, is sufficient to condemn the use of the name *Laverania*. The only argument for the splitting of the genus *Plasmodium* into *Plasmodium* and *Laverania*, and including in the latter genus the æstivo-autumnal plasmodia, is the fact that these plasmodia have crescent-shaped *gametes*, while the *gametes* of the tertian and quartan plasmodia are spherical in shape, but I believe that



this difference in morphology alone is not of sufficient importance to warrant the splitting of the genus, and the confusion that must follow such a procedure, and this belief is shared by the majority of the students of these parasites. Accordingly I have retained all of the malaria plasmodia in the genus *Plasmodium*, believing that this name, as indicating the genus of the parasites, has become so firmly fixed in the nomenclature that it should not be replaced or the genus divided. For more than a quarter of a century the generic name *Plasmodium* has been used by practically all authorities to include all the species of the malaria plasmodia of man, and it should be retained and the name *Laverania* discarded, even though, from a purely zoological standpoint, there may be some justification for its use.

**The Life-cycle of the Plasmodia.**—All of the parasites belonging to the genus *Plasmodium* have an asexual and sexual cycle of existence, the first occurring within the red blood corpuscles of the vertebrate host and the second within mosquitoes belonging to the genus *Anopheles*. In the blood of man the plasmodia live upon and within the red blood corpuscles and are essentially parasites of these cells, eventually destroying them. During this process of destruction of the red corpuscles considerable pigment, known as melanin, is formed from the hæmoglobin of the invaded cell. Man is known as the *intermediate* host of the plasmodia, and the cycle of development of the plasmodia completed in man is known as the *endogenous* or *asexual* cycle.

All species of the malaria plasmodia appear at first within or upon the red blood corpuscles as minute hyaline disks or ring-like bodies devoid of pigment, the *trophozoites*. The forms that are intended to reproduce in man gradually enlarge, develop pigment within them, and just before reproduction occurs practically fill the invaded red corpuscle, which has been almost entirely destroyed during the growth of the plasmodium. These forms are called *schizonts*, and eventually divide, or sporulate, varying numbers of small spores being produced which are called *merozoites*. When sporulation occurs the red blood corpuscle, in which the plasmodium has developed, disappears, and the spores or *merozoites* become free in the blood plasma. The *merozoites* now invade other red blood corpuscles, and the process is repeated. The process of reproduction in the human host is known as *schizogony*, and is asexual in nature.

Among the *merozoites* which are liberated at the time of sporulation there are some that do not undergo *schizogony*, but become differentiated into male and female forms which do not sporulate in man, but, growing at the expense of the red blood corpuscle which they invade and develop in, finally become free in the blood plasma and undergo their further development in the mosquito. The life-cycle in the mosquito is *sexual* in nature, and is known as the *exogenous* cycle, the process of reproduction in the mosquito being called *sporogony*, the mosquito being the *definitive*

host of the plasmodium. The male form which develops in the blood of man is called the *microgametocyte*, and the female form is called the *macrogametocyte*, while these forms are collectively known as *gametes*.

When the blood of a person containing *microgametocytes* and *macrogametocytes* is ingested by the proper species of mosquito, the *microgametocyte* throws out very motile flagella which are finally liberated from the parent body and are known as *microgametes*. At the same time certain changes, consisting of the extrusion of chromatin and other maturation phenomena, occur in the nucleus of the *macrogametocyte*, which prepare it for fertilization, and it is then known as the *macrogamete*. These changes occur normally in the dilated portion of the mid-gut of the mosquito which is called the stomach, and here the *macrogamete* is fertilized by one of the *microgametes* penetrating and fusing with it, the resulting oval or spherical body being known as the *zygote*. I have observed the process of fertilization described in specimens of blood taken upon a moistened glass slide and studied under the microscope.

The *zygote* gradually elongates and becomes motile, and is then called an *oökinete*. The *oökinete* penetrates the epithelial lining of the mid-gut or "stomach" of the mosquito and comes to rest between the epithelial layer and the elastic membrane, where it becomes spherical in shape and forms a cyst, the wall of which is composed of material from the elastic membrane of the insect's stomach. The cyst is called an *oöcyst*, and when completely formed a *sporont*, and the process of *sporogony* begins. The *sporont* develops within it bodies which have usually been interpreted as *sporoblasts*, but which Wenyon (1921) claims have nothing in common with real *sporoblasts*, although they resemble them in morphology. These bodies are really collections of the cytoplasm which later become *sporozoites*. After the development of the *sporozoites* the cyst ruptures and the *sporozoites* make their way to the salivary glands of the mosquito, where they may be found in the cells of the glands, in the ducts, and in the secretions in the ducts, often in immense numbers. When the mosquito bites the salivary secretion containing the *sporozoites* is injected into the wound, and thus the *sporozoites* reach the blood of man, where they invade the red blood corpuscles and become *schizonts*, and the human life-cycle of the plasmodium is initiated.

The development of the malaria plasmodia in the mosquito is completed in from 10 to 14 days, the average period being 10 to 12 days, but it varies somewhat with the different species of plasmodia. The following description, arranged in periods of time, gives an accurate picture of what usually occurs in the insect host during the development of the plasmodia.

*First and Second Days After Ingestion of Malarial Blood.* The fusiform and spindle-shaped *oökinetes* penetrate the epithelial layer of the mosquito's stomach and encyst between this layer and the elastic mem-

brane, the cyst wall probably being formed from the elastic membrane. In stained preparations (Wright's stain) the ookinete presents at, or near, the centre, a deep-red chromatin mass, representing the nucleus, the chromatin being in the form of short, very delicate threads or granules. The pig-

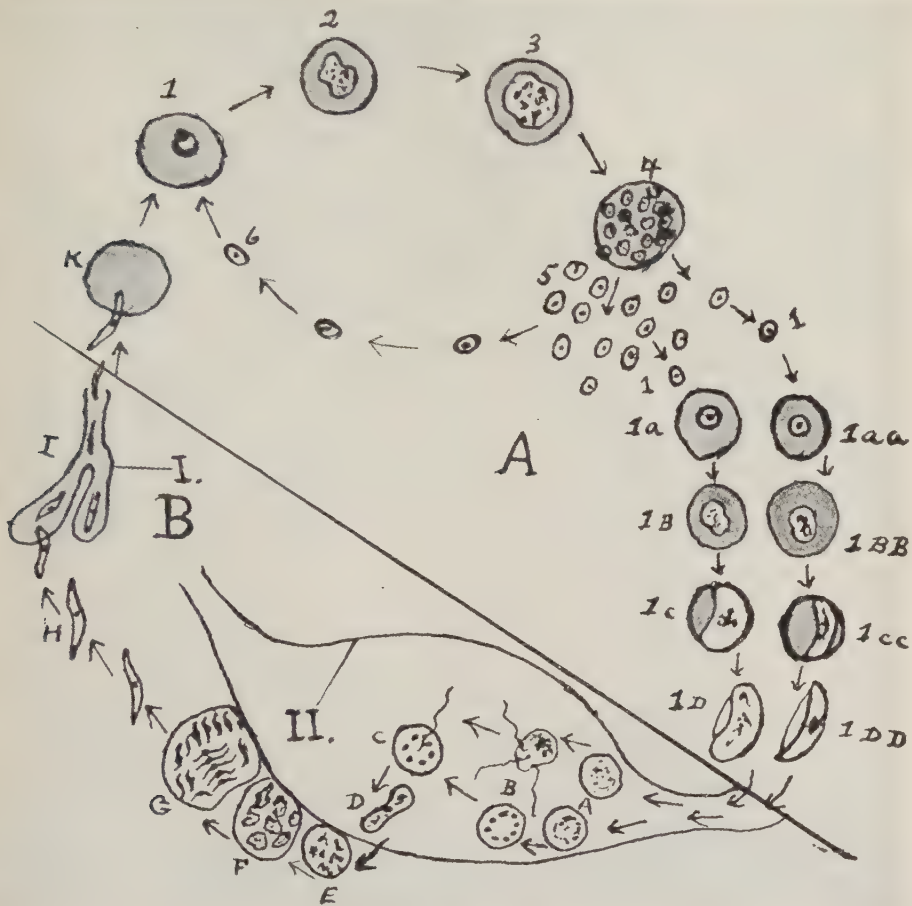


FIG. 65.—Diagram of life-cycle of malaria plasmodium in man and the mosquito. *Ae. vivax*. (Schizogony.) 1 to 6. Life-cycle in blood corpuscles of man. (Sporogony.) A to I. Life-cycle in the mosquito. A. Male and female gametocytes in blood corpuscles of man. A to I. Life-cycle in the mosquito. A. Male gamete flagellated and female gamete prepared for fertilization. B. Male gamete flagellated and female gamete prepared for fertilization. C. Fertilization of macrogamete (female) by microgamete (male). D. Ookinete, the result of fertilization. E, F, G. Various stages in development of the oocyst and the sporozoites within the cyst. H. Free sporozoites in body cavity of mosquito on way to salivary glands. I. Sporozoites in salivary glands of mosquito. K. Sporozoite entering blood corpuscle of man from salivary glands of mosquito when insect bites.

ment is collected in a dense clump at the posterior end or is distributed throughout the cytoplasm; the cytoplasm contains numerous vacuoles and stains a light-blue color. The oöcyst at the end of two days is about the size of a red blood corpuscle, contains considerable pigment, which

is distributed throughout the cytoplasm, while chromatin, stained red, is collected in small granules or minute irregular masses toward the centre of the cyst.

*Third and Fourth Days.* During the third and fourth days the oöcyst increases to twice its original size and develops a well-marked cyst wall. The pigment is not increased in amount and is collected in small masses; the cytoplasm is vacuolated, and the chromatin is distributed throughout it in fine granules or delicate threads.

*Fifth and Sixth Days.* At the end of the sixth day the oöcyst has increased very greatly in size, measuring from 35 to 75 microns in diameter, and projects from the stomach wall outward toward the body cavity of the insect. The cytoplasm appears much vacuolated and granular, refractive, somewhat spherical masses are scattered through it, the so-called *sporoblasts*. The pigment has not increased in amount and, owing to the great increase in the size of the cyst, appears to have greatly diminished, and in some instances to have entirely disappeared. The chromatin is much increased in amount and appears to be collected in the *sporoblasts*, staining bright red, while the cytoplasm stains a pale blue.

*Seventh and Eighth Days.* Under very favorable conditions and in certain species of *Anopheles* the oöcysts, or *sporonts*, have attained their full growth in eight days. At this time a well-marked double outlined membrane surrounds them, which is smooth in contour, while the *sporoblasts* form common centres from which radiate multitudes of delicate, elongated, spindle-shaped, or thread-like bodies, the *sporozoites*. The *sporozoites* originate from the cytoplasm and chromatin of the *sporont*, the entire substance of the organism dividing into multitudes of *sporozoites*. The division occurs in such a manner as to give rise to the radiating clumps which are called *sporoblasts*. When isolated and stained each sporozoite measures from 12 to 15 microns in length, is slender and usually spindle-shaped, and contains one deeply stained mass of chromatin situated near the centre, as a rule, but sometimes more than one mass is present or the chromatin composes a fine fibril situated within the *sporozoite*.

Not infrequently the oöcysts are observed to contain black bodies resembling spores, which were originally believed to be pigment developed within the cysts during the production of the *sporozoites*. In reality, these "black spores," as they were called, are either protozoal parasites of the genus *Nosema*, which infect the cysts, or are produced by the degeneration of the cystic contents.

After the rupture of the cysts and the liberation of the *sporozoites* the latter may be found in practically all of the tissues of the mosquito, but especially in the salivary glands and ducts, and in the salivary secretion. The epithelial cells lining the salivary glands contain the *sporozoites* as well as the ducts of the glands. A period of from one to two days elapses from



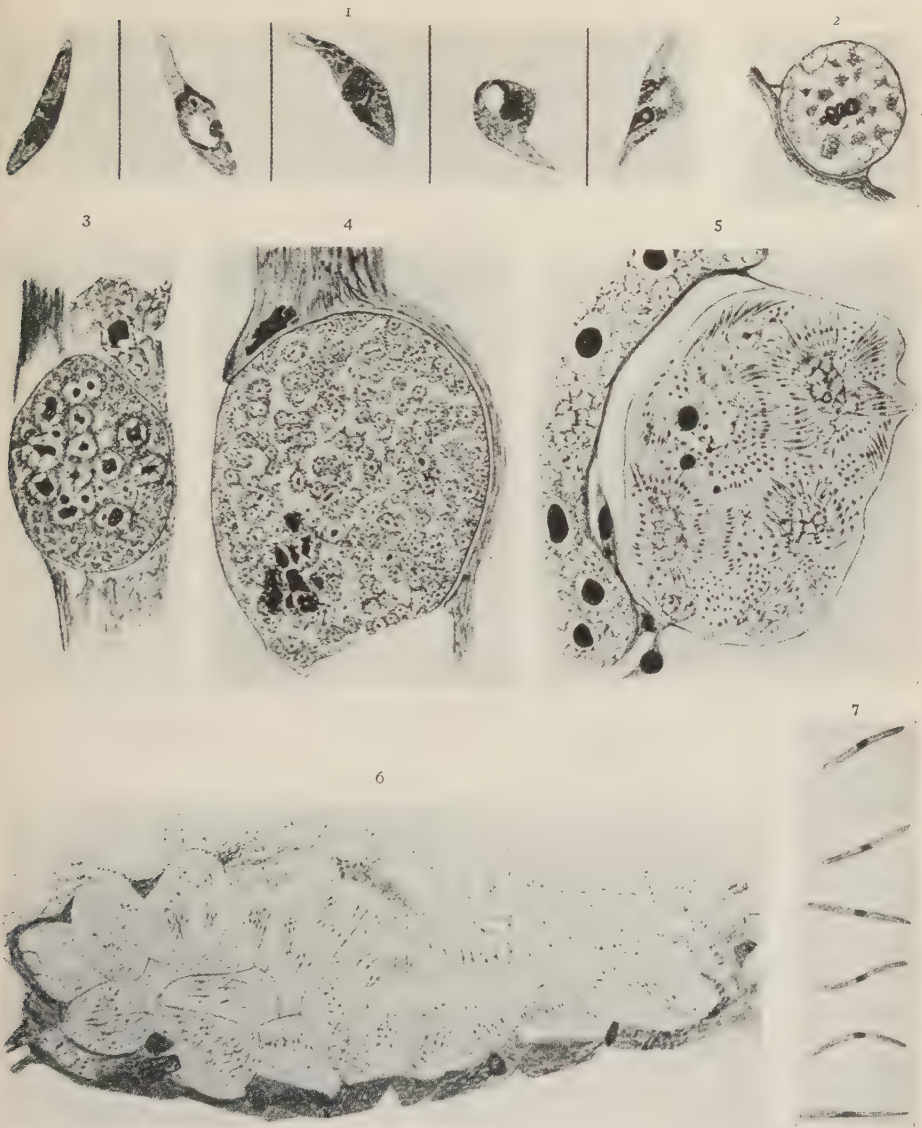


FIG. 66.—Development of malaria plasmodium in mosquito. (*Plasmodium falciparum*.) (After Wenyon, Jour. Roy. Army Med. Corps.) 1. Oökinetes in stomach of *A. maculipennis* twelve and a half hours after feeding. 2. Section through oöcyst showing numerous nuclei. 3. Section through oöcyst showing division of the nuclei. 4. Section through oöcyst in which division of nuclei is complete. 5. Section through oöcyst showing sporozoites. 6. Longitudinal section of salivary gland of *A. maculipennis* containing sporozoites of *P. falciparum*. 7. Sporozoites of *P. falciparum* from salivary gland of mosquito fixed in Schaudinn's fluid and stained with iron hæmatoxylin.

the rupture of the cyst and the liberation of the *sporozoites* to their appearance in the salivary glands.

Wenyon (1921) has studied very carefully the morphology of the development of the malaria plasmodia in the mosquito, and has contributed valuable data regarding the process. The illustrations reproduced of the process are from preparations studied by Wenyon, and give a very excellent idea of the appearance of the various stages of development of the plasmodia in the insect. His observations confirm those of Grassi (1900) and add to them, and to these observers we owe much of our knowledge regarding the subject.

In order that the student may understand the various terms applied to the different stages of the development of the malaria plasmodia in the mosquito the following glossary has been prepared, containing all of the names used in descriptions of the subject.

*Microgametocyte*.—The male organism. In tertian and quartan malarial infections the microgametocyte is spherical in shape, while in æstivo-autumnal infections it is crescentic, and is generally known as a "crescent."

*Macrogametocyte*.—The female plasmodium, spherical in shape in tertian and quartan infections, and crescentic in æstivo-autumnal infections.

*Microgamete*.—The liberated flagellum of the *microgametocyte*.

*Macrogamete*.—The name applied to the *macrogametocyte* after it is ready for fertilization.

*Zygote*.—The organism resulting from the fertilization of the *macrogamete* by the *microgamete*.

*Oökinete*.—The motile free stage of development of the *zygote*.

*Oöcyst*.—The cystic stage of the *oökinete*. Also called a *sporont*.

*Sporoblasts*.—The bodies developed within the *oöcyst*, from which the *sporozoites* are later developed.

*Sporozoites*.—The bodies developed within the sporoblasts which are liberated by the rupture of the *oöcyst*, and which, after reaching the salivary glands of the mosquito, are introduced into the blood of man and begin the human life-cycle of the plasmodium.

*Gametes*.—The name applied to the *microgametocytes* and *macrogametocytes* collectively. Thus the forms of the malaria plasmodia that undergo development in the mosquito are referred to as *gametes*.

**Forms of the Plasmodia Concerned in Relapse.**—The fevers caused by the malaria plasmodia are characterized by their tendency to relapse, and the exact cause of relapse in malaria is still undecided. There are three theories at present concerning the cause of relapse that receive the support of different investigators, *i.e.*, the theory of Ross (1898), that relapses are due to the multiplication of the *schizonts* which are always present in the blood between relapses, but in such small number as to

produce no symptoms; the theory of Schaudinn (1902-03), that relapse is due to the parthenogenesis of the *macrogametes*; and the theory of the writer (1906-07), that relapses are due to the sporulation of resistant forms produced by the conjugation of *merozoites* just after entering the red blood corpuscles, when they are known as *trophozoites*, or "ring-forms."

The theory that relapses are due to parthenogenesis of the macrogametes has been very carefully investigated, and there is no evidence, of scientific value, that supports such a theory of relapse. The theory of Ross is supported by the well-known fact that malaria plasmodia may be present in the blood in small number in the absence of clinical symptoms of the infection, as illustrated in all latent cases, and this theory is most generally accepted at the present time.

The theory that relapses are due to resistant forms produced by conjugation, as advocated by the writer, has the support of morphological evidence of the existence of forms in the blood of relapsing infections which cannot be otherwise explained. The writer described these forms in 1906, and their occurrence in the peripheral blood has been confirmed by W. M. James (1913), and more recently, by S. P. James (1917), and it still remains a question as to their nature, if they are not concerned in the production of relapse.

The greatest objection to the acceptance of this theory has been the lack of evidence in biology that merozoites or spores ever conjugate, but this objection has been removed by the observation of Curtis (1921), who has demonstrated that the zoospores of a *Snychytrium* conjugate in pairs, and that the zygotes so produced are alone capable of developing into resting cells.

For a detailed description of these forms the reader is referred to the contributions of the writer (1906-07), but it may be stated that, when fully developed, they resemble very closely the gametocytes of the malaria plasmodia, and it is the writer's belief that these are the forms which were mistaken by Schaudinn for parthenogenetic macrogametes. S. P. James (1917) has also called attention to the resemblance of these forms to gametocytes, and has stated it to be his belief that they are concerned in the production of relapse.

This theory of the production of relapse explains those infections in which relapse occurs at long intervals, and which it is difficult, if not impossible, to explain by the theory of Ross. The latter theory undoubtedly explains relapses occurring after intervals of a few weeks, but the causation of relapses occurring after many weeks, or months, cannot be thus explained, and deserves further study. In these cases the theory that the relapse is due to a resistant form of the plasmodia that can lie

unchanged in the body for long periods of time best explains the causation of relapse.

For a more detailed description of both the human and mosquito cycle of development of the malaria plasmodia the reader is referred to the description of the various species which follows.

**Species of Malaria Plasmodia.**—The question of the unity or plurality of species among the parasites observed in the blood in malarial infections has always been a matter of controversy since the discovery of the plasmodia by Laveran. At the present time almost every student of the subject believes that there are several species of malaria plasmodia, but there are still some who believe that the species described are merely variants of a very pleomorphic organism. Laveran always believed that there is but one species of malaria plasmodium, and the experience of the various armies with malarial infections during the World War gave some support, in the opinion of several observers, to Laveran's belief. These observers have recorded what they believed to be transmutations of the species that have been generally accepted by protozoologists, and their results have reawakened interest in the question of the plurality of species. While this is so, it must be stated that sufficient evidence has not yet been presented to prove that transmutation of species occurs in the malaria plasmodia, and none of the evidence so far presented is of such a character as to invalidate, in the least, the facts that prove, in the opinion of most qualified observers, the existence of distinct species of malaria plasmodia. The experimental evidence proving that the direct inoculation, into susceptible individuals, of blood containing any of the well-known species of malaria plasmodia is invariably followed by the appearance of the inoculated species, and that species only, in the blood of the inoculated, and the occurrence of the characteristic febrile paroxysm of the species inoculated, is amply sufficient to establish the existence of species among the human plasmodia, and when to this proof is added the evidence of the results of more than 100 mosquito experiments which are recorded in the literature, in which the species of plasmodia obtained by the mosquitoes from the infected individual invariably appeared in the blood of individuals bitten by the insects, accompanied by the characteristic clinical symptoms usually produced by the species, the proof of the plurality of species is, I believe, incontrovertible.

Gerhardt (1884) was the first to successfully produce malarial infection in man by the direct inoculation of blood containing tertian plasmodia, and in 1889, Antolisei and Angelini produced typical tertian infection in man by the inoculation of blood containing the tertian plasmodium, while in the same year Gualdi and Antolisei produced quartan and æstivo-autumnal infections by the inoculation of blood containing the quartan and æstivo-autumnal, respectively. These pioneer investigations were soon



confirmed by others, and in 1910, Ross published the details of no less than 51 cases of malaria produced by the inoculation of blood from patients suffering from the various forms of malarial infection, which he had collected from the literature. In every instance the species of plasmodium inoculated appeared in the blood of the inoculated individual, and that species only.

The inoculation of the blood of malarial patients containing only the sexual forms, or *gametes*, has invariably been followed by negative results. Thayer (1897) inoculated intravenously into a healthy man several c.c. of blood from a patient whose blood contained only the *gametes* (crescents) of æstivo-autumnal plasmodia, and no infection resulted. Elting (1899) inoculated three individuals with from 3 to 3.5 c.c. of blood containing only crescents with a negative result in each instance. These experiments have been repeated by others, and it may be stated that the inoculation of the sexual forms, or gametes, of the various species of malaria plasmodia is never followed by infection in the inoculated individual.

The recent efforts to treat paresis by the inoculation of malaria plasmodia has only strengthened the proof of the plurality of species among the malaria plasmodia. Doerr and Kirschner (1922) passed the same strain of the tertian plasmodium (*P. vivax*) through 62 individuals during 13 consecutive months, 240 generations of the plasmodium occurring during this time. There was not the slightest change in the morphology of the plasmodium during the entire period, the species breeding true in every generation. Wagner-Jauregg (1922) found that in inoculating *P. vivax* for the treatment of paresis there was no change in the morphology of the plasmodium in 37 transmissions in direct descent, and that the same was true of the total 200 transmissions made by him, the only species of plasmodium appearing in the blood of the inoculated being *P. vivax*. Muehlens and Kirschbaum (1923) during 15 consecutive months transmitted the same strain of *P. vivax* to 59 individuals; *P. malaria*, the quartan plasmodium, to 5 individuals; and *P. falciparum*, the tertian æstivo-autumnal plasmodium, to 9 individuals, and in no case was there any evidence of mutation of species, each species breeding true morphologically and clinically.

At the present time there are three species of malaria plasmodia causing disease in man that are recognized by practically all students of the subject. These are *Plasmodium malarie*, Marchiafava and Celli, 1885; *Plasmodium vivax*, Grassi and Feletti, 1890; and *Plasmodium falciparum*, Welch, 1897. All three species were undoubtedly observed by Laveran, in 1881, but he did not recognize their specific differences, and consistently refused to do so, despite all proof to the contrary.

While practically all authorities accept these three species of malaria plasmodia as distinct, numerous observers believe that more than one

species or variety of plasmodium is concerned in the etiology of the æstivo-autumnal fevers, and that *Plasmodium falciparum*, the accepted cause of these fevers, is really divisible into species or subspecies. Thus Grassi and Feletti (1890) recognized two species of æstivo-autumnal or pernicious plasmodia; Mannaberg (1893) and Manson (1907) three species, a pigmented quotidian, an unpigmented quotidian, and the malignant tertian; while Marchiafava and Bignami (1892) recognized two species, a quotidian and tertian æstivo-autumnal plasmodium.

In 1901, as the result of the study of hundreds of malarial infections contracted in Cuba and the Philippines, I accepted Marchiafava and Bignami's classification, and described (1901) two species of plasmodia associated with the æstivo-autumnal infections that I studied, one sporulating in twenty-four hours and causing a quotidian fever, the other sporulating in approximately forty-eight hours and causing a peculiar type of tertian fever. Further study of these two species convinced me that the evidence was not sufficient to entitle the quotidian plasmodium to specific rank, and in 1909 I proposed that it be regarded as a subspecies of *Plasmodium falciparum*, and gave to it the name, *Plasmodium falciparum quotidianum*.

In descriptions of the æstivo-autumnal plasmodia, published at various intervals (1907, 1909, 1914, 1919), I have shown that *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* are distinguishable morphologically, differ in the time consumed in their human life-cycle, and produce characteristic febrile paroxysms. The majority of text-book writers do not accept more than one species of æstivo-autumnal plasmodium, although they admit that clinically both the tertian and quotidian types of fever occur. They explain the occurrence of these clinical types by asserting that *Plasmodium falciparum* sporulates at irregular intervals, at one time in twenty-four hours, and at another in approximately forty-eight hours, an assumption that is at variance with the biological laws regarding sporulation as illustrated in the other species of malaria plasmodia. *Plasmodium malariae*, the quartan plasmodium, does not sometimes sporulate in thirty hours, and sometimes in seventy-two hours, but always in approximately seventy-two hours; nor does *Plasmodium vivax*, the benign tertian plasmodium, sometimes sporulate in twenty-four hours, and sometimes in forty-eight hours, and to claim that *Plasmodium falciparum* is so marked an exception to the rule, as illustrated by sporulation in the other malaria plasmodia, is illogical and simply begs the question. Those who believe in only one species of malaria plasmodium rightly use the same argument, claiming that under certain conditions the plasmodium sporulates in twenty-four hours, in forty-eight hours, or in seventy-two hours, accompanied by change in its morphology. Biologically such a variation in the time of reproduction of a single species is not in accordance

with the evidence, and the assertion that *Plasmodium falciparum* sporulates sometimes in twenty-four hours and sometimes in forty-eight hours, producing each time a different number of merozoites and a different type of febrile paroxysm, is no more worthy of belief than that there is but one species of malaria plasmodium with three different life-cycles in man, as shown by the variations in the time of sporulation.

Many careful students of the malaria plasmodia believe that at least two varieties or species of *Plasmodium falciparum* exist, and the trend of recent scientific opinion is in favor of the recognition of more than one species of this plasmodium. Bass (1920), who first cultivated the malaria plasmodia, and who has had the advantage of studying the morphology and development of the æstivo-autumnal plasmodia in pure cultures states: "that there are at least two and probably more subdivisions of the æstivo-autumnal parasite." He accepts the tertian and quotidian species and describes their morphology and development in cultures. In a personal communication he writes that he feels certain that there are two distinct species of æstivo-autumnal plasmodia, differing in their morphology, as observed in preparations of the blood, and retaining these differences during their development in cultures. It may therefore be stated that there are two plasmodia concerned in the etiology of æstivo-autumnal malaria, *Plasmodium falciparum* and *Plasmodium falciparum quotidianum*.

Besides the four species of plasmodia mentioned other species have been described from time to time, of which two possess some claim to specific recognition. These are *Plasmodium vivax*, variety *minuta*, Emin, 1914, and *Plasmodium tenue*, Stephens, 1914.

The plasmodium described by Ahmed Emin, in 1914, was found by him in the blood of patients in hospital at Camaran, an island in the Red Sea. He regarded this plasmodium as a subspecies or variety of the benign tertian plasmodium, and called it *Plasmodium vivax*, var. *minuta*. In 1900, I published the description of a plasmodium observed in the blood of American soldiers returning from the Philippines which was undoubtedly identical with the plasmodium described by Emin, but did not name it, as I was not sure that it was a distinct species or subspecies, stating: "I believe that this parasite is either a distinct variety of the malaria plasmodia, or that it is a tertian parasite which has acquired the characteristics described through some unknown condition of environment acting on the development of the organism."

More recently Stephens (1922) has described a plasmodium in a patient contracting malaria in East Africa which agrees in morphology with *Plasmodium vivax*, var. *minuta*, and which he has named *Plasmodium ovale*. If this plasmodium is identical with that of Emin, as seems most probable, the name *ovale* will have to become a synonym of "*minuta*," the proper name of the plasmodium, if it be regarded as a distinct species,



being *Plasmodium minutum*, Emin, 1914. It is my belief that the plasmodium described by Emin is a distinct variety of *Plasmodium vivax*, and that until it can be proven that it is entitled to full specific rank it should be regarded as a subspecies and Emin's name, *Plasmodium vivax*, var. *minuta*, be retained.

*Plasmodium tenue* was described by Stephens, in 1914, who found it in a blood smear sent to him by Major Kenrick, I. M. S., from Pachmari, Central Provinces, India. His observations have recently been confirmed by Sinton (1922), and both observers believe that *Plasmodium tenue* is a distinct species. Further observations are needed before their opinion can be accepted, and *Plasmodium tenue* must still be regarded as a doubtful species.

Several other species of malaria plasmodia have been described, but further study has shown that they are all identical with one of the four first-mentioned species, and the various names that have been given them become synonyms of the accepted species.

However, it is well to remember that there may be species of malaria plasmodia that have not as yet been described, and the clinical characteristics of malarial infection in different localities, presumably due to the recognized species, lend some support to this theory, as it is well known that infections vary greatly in severity, and that in some localities nearly every infection is pernicious, while in others pernicious symptoms do not develop, although the same plasmodium is presumably the cause of both.

**Cultivation of the Malaria Plasmodia.**—Coronado (1892) was the first to claim the successful cultivation of the malaria plasmodia in unsterilized water. He described the development of the plasmodia in twenty-four hours after placing infected blood in the water. His work was repeated by numerous observers and never confirmed, and we now know that he must have mistaken some protozoon in the grossly contaminated water for the malaria plasmodia.

Sacharoff (1890) found that in malarial blood obtained by leeches the plasmodia remained alive for over a week, provided the leeches were kept upon ice, but that no reproductive changes occurred during this time. He found that the æstivo-autumnal plasmodium remained actively amœboid for seven days, and capable of infection for four days, but that under such conditions the tertian plasmodium remained amœboid for only forty-eight hours. His results were confirmed by Rosenbach and Blumer. Thayer (1897) quotes some experiments of Hamburger and Mitchell with leeches in which they were able to keep the æstivo-autumnal plasmodium alive in the leech for over a week, and the tertian plasmodium for ten days, during which time there was some evidence of development, as shown by the appearance of pigment in the æstivo-autumnal plasmodia.

Bass (1911) was the first to successfully cultivate malaria plasmodia



and to observe in cultures the entire human cycle of development. In his first paper the technique of cultivation was not given because it was imperfect and often unsuccessful, but in 1912, in conjunction with Johns, Bass published his technique, and his results have been confirmed by numerous investigators. He has been successful in cultivating *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium falciparum quotidianum*. The exact method of cultivation employed by Bass and Johns is given in detail in the Appendix, but briefly, it consists in the addition to blood drawn from the patient's vein of one-tenth c.c. of a 50 per cent. solution of dextrose, after which the blood is defibrinated and placed in tubes, care being taken to see that the column of blood serum is from 1.25 to 2.5 cm. deep over the blood cells after the latter have settled to the bottom of the tube. Development of the plasmodia occurs at the top of the column of precipitated cells in a layer varying in thickness from 0.05 to 0.1 cm. Bass was successful in cultivating as many as five generations of plasmodia, but usually the cultures die out after from two to three generations have been cultivated. Bass believes that with proper technique and great care the cultures might be prolonged indefinitely, but experience has shown that it is practically impossible to carry the plasmodia through more than three or four generations, and that then all growth ceases. In fact it has been the experience of many observers who have attempted to cultivate the plasmodia, that while it is comparatively easy to cultivate one generation, most of the cultures die out after two generations have developed. This would be expected, as the plasmodia develop in cultures within the red blood corpuscles, as in man, and these cells soon degenerate in the cultures. Therefore, it is necessary to transfer the culture into tubes containing fresh blood cells and serum, but even when this is most carefully done the cultures die, and it is rare to secure more than three generations of the plasmodia. This fact has militated greatly against the usefulness of the cultures for teaching purposes and in research, but even so, the success of Bass and Johns in cultivating the malaria plasmodia *in vitro* was an epoch-making addition to our knowledge of these parasites. It is more than probable that further research will develop methods which will render it possible to cultivate the plasmodia indefinitely, and already the cultures have been made use of in preparing an antigen used in a complement fixation test in the diagnosis of malaria.

Lavinder (1913) in the United States, and Thompson and Thompson (1913) in England, were the first to confirm the work of Bass and Johns. Lavinder was successful in cultivating *Plasmodium falciparum*, and Thompson and Thompson cultivated *Plasmodium vivax*. Ziemann (1913) was also successful in cultivating both *Plasmodium vivax* and *Plasmodium falciparum*, and since that time numerous investigators have succeeded in cultivating these parasites. Recently Sinton (1922) has described a

simplified method of cultivation (see Appendix) which he has used with success, and which appears to be of practical value.

In cultures only the forms of malaria plasmodia concerned in the human life-cycle, or *schizogony*, undergo development, the gametes, if present, undergoing no reproductive changes or presenting any of the phases of development that occur normally in the middle intestine of the mosquito. If parthenogenesis occurs in the malaria plasmodia no evidence of it has been secured from cultures, and no resting or resistant forms of the plasmodia have been described as developing in the cultures.

**Morphology of the Malaria Plasmodia.**—As the morphology of the various species of malaria plasmodia differs considerably it has been thought best to discuss this subject in the consideration of each species rather than to attempt to give a composite picture of the morphology. Here it may be stated that all species of the plasmodia appear first within or upon the red blood corpuscles as minute hyaline bodies which gradually increase in size, develop pigment, and finally fill more or less of the invaded corpuscle which has undergone characteristic changes produced by the growth of the parasites. After attaining full size all of the plasmodia divide into minute oval or almost round bodies, varying in number with each species, which are liberated by the dissolving of the degenerated red blood cell, and which attack new red corpuscles and repeat the cycle of development. At all stages of development the plasmodia are colorless, consisting of hyaline cytoplasm containing a vesicular nucleus and pigment derived from the hæmoglobin of the host cell. In addition to the forms described other forms are observed which do not divide when fully developed, but fill the host cell and undergo no further development in the blood of man. These are the *gametes*, which undergo development in the middle intestine of the mosquito. The *gametes* are colorless and composed of hyaline cytoplasm containing a nucleus and pigment, but can be easily differentiated from the forms concerned in the human life-cycle when stained by various methods, as will appear in the description of the various species.

**Method of Transmission.**—Until very recent times it was held by students of malaria that the infection could be transmitted to man through the air or by water. The arguments in favor of these methods of transmission were at the time unanswerable, in the opinion of scientists, and even after the discovery of the plasmodia by Laveran it was believed that these parasites reached the blood of man either through breathing infected air or drinking infected water. At the present time we know that the only method of transmission of these parasites to man that has been scientifically demonstrated is by the bite of infected mosquitoes, and one is justified in stating that, under natural conditions, the malaria plasmodia can only be transmitted from man to man through the agency of these

insects. While it is possible to transmit the plasmodia to man by the inoculation of blood from patients infected with the parasites, such a method of infection probably never occurs in nature. The direct mechanical transmission of the plasmodia by mosquitoes that have bitten patients whose blood contained numerous plasmodia might be possible, but is certainly very improbable, and there is not a single case of record in which malarial infection was conveyed in this manner.

The conception that mosquitoes transmit malaria is by no means a recent one, such a theory having been held by the early Roman observers, Varro, Columella, and Vitruvius. Even in regions inhabited by savage peoples the belief that mosquitoes are responsible for the fevers present in such regions is prevalent, and Koch stated that in German East Africa the native name for malaria, mbu, was also the native name for the mosquito, and that the natives firmly believed that the fever was due to the bite of these insects. In 1848, Nott, of Mobile, in a contribution upon yellow fever, speaks of the transmission of malaria by the mosquito as a fact, and in 1883, King, of Washington, advocated this theory vigorously, and collected a great mass of evidence tending to show that malaria is transmitted by mosquitoes. After the appearance of King's paper numerous writers published arguments in favor of the transmission of the plasmodia by the mosquito, chief among whom may be mentioned Laveran, in 1884; Flugge, in 1891; Pfeiffer, in 1892; and Manson, in 1894. In 1898, in his Goulstonian Lectures, Manson directed attention anew to this subject, and while some of his deductions have been proven to be erroneous, it is unquestionably true that to this investigator we owe the stimulation of interest which resulted in the discovery of the true relation of the mosquito to the malarial fevers. Manson stated in these lectures that he believed that the crescentic and flagellated parasites are the extracorporeal homologues of the intracorporeal sporulating plasmodia, and that, as the mosquito has been proven a host for *Filaria nocturna*, the embryos of this worm being removed from the blood of man by the insect and then developing within it, so the same insect might remove these extracorporeal bodies of the malaria plasmodia and constitute the host for these particular forms of the plasmodia. At that time Manson did not believe that the mosquito inoculated malaria into man, but that the insect removed from man certain stages of the plasmodia which afterward underwent development in the mosquito and were then liberated in the water or dust and thus infected man.

To Ross, a Surgeon Major of the Indian Army Medical Service, we owe the discovery of the real relation of the mosquito to the malarial fevers. Stimulated by Manson's contributions, this investigator, in 1895, studied the development of the plasmodia causing æstivo-autumnal malaria in the mosquito, and proved that the crescents (*gametes*) underwent definite changes in the middle intestine of the mosquito, flagella developing



in the same manner as observed after the withdrawal of blood containing the crescents from man. In 1897, Ross described the large cystic bodies situated in the outer layers of the wall of the middle intestine of the mosquito and considered that these were developmental forms of the plasmodia, and that he had at last succeeded in finding the mosquito in which the malaria plasmodia underwent their extracorporeal cycle of development.

In 1898, Ross studied the relation of the mosquito in the transmission of bird malaria, due to *Proteosoma*, and proved that this form of malaria is transmitted by mosquitoes belonging to the genus *Culex*. He found that in mosquitoes which had been allowed to bite birds infected with *Proteosoma*, large pigmented bodies developed in the wall of the middle intestine similar to those he had previously observed in mosquitoes which had bitten malarial patients. He found that these bodies increased in size until they protruded from the wall of the intestine, and that there developed within them a large number of delicate thread-like bodies, which, after rupture of the cyst in which they had developed, were liberated in the body cavity of the insect. He was able to trace these bodies to the cells and ducts of the salivary glands of the insect, and he then advanced the theory that the thread-like bodies were injected into the bird when the mosquito bit, and that these bodies began anew the life-cycle of the parasite in the bird. He proved this theory experimentally by allowing infected mosquitoes to feed on healthy birds, and obtained 22 successful results in 28 sparrows thus experimented with, all of which showed large numbers of proteosoma, in their blood in from five to eight days after being bitten by infected mosquitoes. Ross was thus able to prove beyond question that the malaria of birds is transmitted by the mosquito, and he stated it as his belief that what had been found true of bird malaria would also be found true of human malaria.

The credit for the experimental demonstration that mosquitoes transmit malaria to man belongs to the Italian investigators, Bignami, Bastianelli, and Grassi, who, following the work of Ross, endeavored to produce malaria in man by allowing infected mosquitoes to bite healthy individuals. In 1898 these investigators were successful in producing a double tertian malarial infection in man through the bites of infected *Anopheles* mosquitoes, and in February, 1899, they were successful in infecting *Anopheles maculipennis* with the quartan plasmodium (*Plasmodium malariae*) and were able to trace the developmental stages of this species of plasmodium in the mosquito. They were also successful in transmitting the benign tertian plasmodium, *Plasmodium vivax*, to a second individual through the bite of the infected *Anopheles*, and in transmitting *Plasmodium falciparum*, the æstivo-autumnal plasmodium, to man in this manner. In the same year Bastianelli and Bignami reported three



successful transmission experiments by *Anopheles*, the first, a double tertian infection; the second, a single tertian infection; and the third, an infection with one of the æstivo-autumnal plasmodia. These investigators determined that only mosquitoes belonging to the genus *Anopheles* are capable of transmitting the plasmodia causing human malaria, while only mosquitoes belonging to the genus *Culex* transmit bird malaria. The entire life-cycle of the plasmodia within the mosquito was worked out by these observers for all species of plasmodia, and corresponded in almost every detail with the life-cycle of *Proteosoma* in the mosquito, previously described by Ross.

That the cycle in the mosquito is sexual in nature was demonstrated by MacCallum (1897), who, in studying the development of *Halteridium*, observed that the fully developed extracellular halteridia consisted of two forms, one of which was flagellated, the other non-flagellated. He observed that the flagella, breaking away from the flagellated form, penetrated the non-flagellated organisms, and that after penetration a motile body, or *zygote*, resulted. The same phenomena were noted in the development of the human malaria plasmodia in the mosquito by Grassi, Bastianelli, and Bignami, and were soon confirmed by numerous investigators.

Since the work of the Italian observers mentioned many investigators have been successful in experimentally transmitting malaria to man by the bites of infected *Anopheles*, and all species of the plasmodia have been so transmitted with the exception of *Plasmodium vivax*, var. *minuta*, and *Plasmodium tenue*. At the present time all authorities accept the mosquito transmission of malaria, and believe that this is the only way in which the malaria plasmodia are transmitted in nature.

There are many factors governing the transmission of the infection which are of great importance, and which must be understood if one is to have a clear idea of the epidemiology of this most important group of protozoan infections. Some of the most important of these factors will now be considered.

The factors influencing the transmission of malaria plasmodia by the mosquito have to do with both man and the mosquito. It does not always follow that because individuals infected with the plasmodia, and mosquitoes of the genus *Anopheles*, are present in a locality that the locality is badly infested with malarial fevers, for both man and the mosquito may be present and yet there may be little or no malaria, as will be understood if the many factors governing the transmission of the plasmodia are considered. The most important of these factors are: enough "carriers" of the plasmodia to infect mosquitoes; "carriers" with enough *gametes* to infect mosquitoes; proper age of the *gametes*; a proper proportion of the male and female *gametes*; suitable species of *Anopheles*; proper local conditions for

the life of the mosquito during the period required for the development of the plasmodia into *sporozoites*; proper temperature for the development of the plasmodia in the mosquito; and access of the infected mosquito to a non-immune population.

In order that mosquitoes become infected with malaria plasmodia there must be a sufficient number of human beings present in the locality whose blood contains *gametes*, and in order that the infection may be transmitted to man by the mosquito it is necessary that the gametes develop to maturity in the mosquito and that the insect bite a non-immune individual. This sequence of events may be interrupted at several points, and the transmission of the plasmodia rendered impossible. Under ordinary conditions such interruptions occur very frequently, and malaria in such regions is of sporadic or endemic character, but sometimes all conditions are favorable to the transmission of the plasmodia, and at such times we have veritable epidemics of the malarial fevers.

#### Factors Concerning the Development of the Plasmodia in Man.—

It is well known that after an attack of malarial fever has persisted for from 8 to 10 days or more, and quinine has not been given in sufficient dosage to control the symptoms of the infection, there develop in the blood of the infected individual forms of the plasmodia which are capable of infecting anopheline mosquitoes, and which are known as *gametes*. Such an individual is called a "carrier" of malaria, and in order for the infection to be transmitted by the mosquito enough of these insects must be infected to render the chances of one of them biting a non-immune individual certain. If only one or two mosquitoes bit a single carrier of the plasmodia the chances of either surviving and biting a non-immune individual would be very small, but if hundreds of mosquitoes had access to scores of "carriers," the chances of many of the insects biting non-immune individuals would be very great, and the infection would without doubt be thus transmitted. It is therefore necessary that in any locality where anopheline mosquitoes are present there be enough "carriers" present to infect a considerable number of mosquitoes if these insects are to transmit the infection to the non-immune population. The percentage of individuals showing *gametes* in their blood, and who are thus "carriers" of the plasmodia, varies greatly in different localities, but it may be stated that, unless properly treated, practically 80 per cent. of all individuals who have developed symptoms of malarial infection will probably become "carriers" and capable of infecting mosquitoes. The number will vary, of course, owing to conditions favoring the persistence of the infection, the type of infection present, and other conditions with which we are still unfamiliar. This subject has been most carefully studied in the æstivo-autumnal infections, and it has been shown that the incidence of *gametes* in the peripheral blood cannot be taken as a true index of their

actual occurrence in the body, for in many instances it has been demonstrated that blood obtained by splenic puncture was rich in *gametes* when none could be demonstrated in the peripheral blood, and in such instances the *gametes* may appear in the peripheral blood at any time, and thus be available to the mosquito.

In my own experience *gametes* have been observed in the peripheral blood in a little over 33 per cent. of all cases of æstivo-autumnal malaria that I have examined, numbering many hundreds, but I am convinced that a longer search would have revealed a greater number of "carriers."

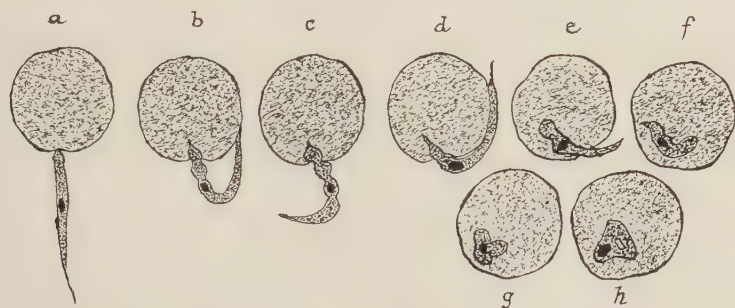


FIG. 67.—Figures illustrating the entrance into red blood corpuscle of the sporozoite of *Plasmodium vivax* and development of trophozoite. (After Schaudinn.) a, b, c and d. Sporozoite attempting to enter the red blood corpuscle. e. Sporozoite half way in the red blood corpuscle. f. Sporozoite wholly within the red blood corpuscle, showing contraction in length and increase in breadth of the organism. g and h. Trophozoite developed from the sporozoite.

It is a conservative statement that a fraction over 50 per cent. of æstivo-autumnal infections show *gametes* in the peripheral blood. In the instance of recurrent infections in Filipinos *gametes* occurred in fully 80 per cent., and were as numerous in the adult Filipino as in the children. As most of these individuals had received little or no treatment, I believe that this percentage is true of untreated infections, and that 80 per cent. of such infections will show *gametes* and become "carriers" of the plasmodia.

There is little data available regarding the percentage of patients infected with *Plasmodium vivax* and *Plasmodium malarie* in whom *gametes* develop, but in my own experience, these forms have been found in about 50 per cent. of the patients admitted to hospital, and in about 30 per cent. of latent infections.

Not only must *gametes* be present in the peripheral blood of man in order that mosquitoes become infected and transmit the plasmodia, but there must be enough *gametes* present to infect the mosquito. Darling (1910) has shown that the peripheral blood of man must contain at least 12 *gametes* per cubic centimetre, or more than 1 to 500 leucocytes, in order to be infective to the mosquito and that patients whose blood contains that number should be regarded as "carriers." Darling's conclusions have been confirmed by Thompson (1912) and others, and demonstrate that

individuals may have *gametes* in their blood and still be unable to infect mosquitoes.

The transmission of the plasmodia by the mosquito is further restricted by the fact that there must be the right *proportion existing between the number of male and female gametes (microgametocytes and macrogametocytes)* in the peripheral blood of man for infection of the mosquito to result. Usually female gametes are more numerous than male, and when not too numerous this condition offers the best chances for infection. Sometimes the most careful examination will not result in finding any male gametes, and when this occurs the blood is not infective to the mosquito. Stephens and Gordon (1924) found the proportion of females to male as 3 to 1; Knowles (1919) as 10 to 1; and Abrami and Senevet (1920) to vary from 2 to 1 to 3 to 1. In my own experience I have found the usual proportion of females to males to be from 3 to 1 to 4 to 1, and that it varies considerably with the different species of plasmodia, which may account for the relative rarity of certain species.

Another factor that affects the transmission of the plasmodia by the mosquito is the *age of the gametes*, as it has been demonstrated that in most instances the gametes are not infective to the mosquito when they first appear in the blood, but that they must be about 12 days old before they are capable of undergoing their development in the insect. This fact is of great importance in prophylaxis, as the proper treatment of infections may prevent patients from becoming true "carriers" of the plasmodia by destroying the gametes before they are capable of infecting the mosquito.

**Factors Concerning the Development of the Plasmodia in the Mosquito.**—In addition to the factors influencing transmission already mentioned there are numerous conditions which influence the development of the plasmodia in the mosquito, or have to do with the insect itself, which have a profound effect upon the transmission of the infection to man.

As already stated, the *gametes* of the malaria plasmodia of man only develop in mosquitoes belonging to the genus *Anopheles*, and where there are no anopheline mosquitoes malaria plasmodia cannot be transmitted to man. Not only is this true, but, as will be seen later, only certain species of anopheline mosquitoes are common transmitters of the plasmodia, and unless such species are present in a locality there is little malaria, although human "carriers" may be present in considerable numbers.

*Temperature* has much to do with the transmission of the plasmodia, for unless the temperature is favorable the *gametes* will not develop in the mosquito, as has been proven by numerous investigators. Jancso (1905) determined that the lowest temperature at which the malaria plas-



modia would develop in *Anopheles maculipennis* was between 15 and 16° C. (59 to 61° F.) and that 53 days are required for the production of *sporozoites* of *Plasmodium vivax* at this temperature. Wenyon (1921) found that temperatures between 9.6° C. and 18.2° C. (48.5 to 64.5° F.) prevented development. Thompson and Woodcock (1923) state that the optimum temperature for the development of *Plasmodium vivax* in the mosquito is 25° C. (77° F.) and complete development occurs in 11 days, while for *Plasmodium malariae* the optimum temperature is 22° C. (72° F.) and development is completed in 18 to 21 days. They also found that for *Plasmodium falciparum* the optimum temperature is 30° C. (86° F.), and complete development occurs in from 10 to 11 days. They state that at a temperature of 25° C. (77° F.) *Plasmodium vivax* requires 11 days for complete development, and *Plasmodium falciparum* requires 14 days. In the Balkans, Wenyon found that *Plasmodium vivax* required 40 days to develop *sporozoites* in the mosquito at a temperature of 17° C. (62° F.).

It will be noted that the optimum temperature for development in the mosquito varies for each species of plasmodia, and that there is a difference in the number of days required for the development of *sporozoites*. It has been found that while low temperatures will prevent the development of the malaria plasmodia in the mosquito, that even long-continued low temperatures will not always kill the plasmodia, and that subsequent development of the plasmodia will occur when the temperature again becomes favorable. King (1917) found that *Plasmodium vivax* in *Anopheles quadrimaculatus* will withstand a temperature of 0° C. (32° F.) for four days, and a temperature between 7 and 8° C. (45 to 47° F.) for 17 days, and that *sporozoites* developed from the oöcysts after exposure to these temperatures. He also determined that *Plasmodium falciparum* could withstand a temperature of 1.7° C. (35° F.) for 24 hours.

These observations demonstrate that development of the plasmodia may begin in the mosquito, be interrupted for a period of time by unfavorable temperature conditions, and then be completed when the temperature again becomes favorable, and suggest that the plasmodia may exist in hibernating mosquitoes, and that in this manner malarial infection is carried over the winter season. Mitzmain (1916) examined 1,211 hibernating mosquitoes for plasmodia early in the spring, and did not find a single one showing the parasites. Wenyon (1923) kept infected mosquitoes at temperatures corresponding to winter temperatures for different periods of time, after which the insects were kept at temperatures favoring the development of the plasmodia, and found that the low temperatures to which the insects were exposed did not prevent the development of the plasmodia when the temperature was again favorable, and that the plasmodia under unfavorable temperature conditions remained dormant for

weeks and even months. He believes that his results prove that, under certain conditions, it is possible for the malaria plasmodia of man to remain alive in hibernating mosquitoes through a winter and renew their development in the insect in the spring.

At the present time it cannot be said that it is proven that in nature the plasmodia live through the winter in hibernating mosquitoes, but it is very probable that they do so under favorable conditions, where low temperatures are not too extreme. It is also very probable that certain stages of the life-cycle of the plasmodia in the mosquito are more resistant than others, and that unless development has progressed to the more resistant stage or stages the plasmodia perish. If this is true it will explain some of the diverse results obtained by different observers as to the effect of low temperatures upon malaria plasmodia in infected mosquitoes.

Under favorable conditions the malaria plasmodia may live for considerable periods of time in mosquitoes kept in captivity. Thus Mayne (1922) found the plasmodia in mosquitoes kept for 70, 71, 83, and 92 days, respectively, five *Anopheles punctipennis* being used. These insects were allowed to bite a patient showing gametes of *Plasmodium falciparum* in his blood, once only, then kept at room temperature for six days, and afterward in a container registering from 44 to 78° F. In all of the insects, after the periods mentioned, the salivary glands were found to contain sporozoites. Mayne also found that a mosquito infected 55 days previously was capable of transmitting the plasmodia to a human host, but that mosquitoes infected 61, 66, and 67 days previously did not transmit the plasmodia.

There is no evidence that infection with the malaria plasmodia is hereditary in the mosquito.

The numerical prevalence of certain species of anopheline mosquitoes has much to do with the transmission of the plasmodia. It is obvious that the greater the number of suitable anopheles and human "carriers" of the plasmodia, the greater will be the chances of the transmission of the infection. However, this does not always follow, for there may be present in the locality a more or less immune population, temperature conditions may be unfavorable, or other factors may be present that interfere with the transmission of the plasmodia. The presence of numerous anopheline mosquitoes is of no importance if there are few or no human "carriers" of the plasmodia present in the locality.

It should be remembered that a few infected mosquitoes may transmit the plasmodia to many individuals, for one infected insect may bite many human hosts and infect them. In fact, in most malarial localities the percentage of mosquitoes showing malaria plasmodia is very much smaller than would be expected.

The percentage of infected mosquitoes is of much importance in the

transmission of the plasmodia to man, and the greater the percentage, provided a non-immune population is present, the greater the number of transmissions, and in this way veritable epidemics of malarial disease may occur. An instance of recent times is the great epidemic of malaria that swept through the southern region of Russia following the World War, in which, in regions in which malaria was previously only of sporadic occurrence, thousands of infections occurred within a short time, and entire populations became infected.

Considering the number of human "carriers" of the plasmodia that are present in most malarial regions, and the chances of infection of suitable mosquitoes, the percentage of insects that show the plasmodia is generally remarkably low. Thus in Italy, Celli (1913) found only 2.5 per cent. of the anopheline mosquitoes that he examined infected; A. Plehn (1904) in very malarial regions in Africa found only 2.3 per cent. of *Anopheles* infected; while the Sergents (1909), in Algeria, found 1.6 per cent. infected.

While it is generally true that only a small percentage of anopheline mosquitoes show infection with the plasmodia, it is also true that in some badly infected localities a considerable percentage of these insects are found infected with the plasmodia. Ziemann (1906), in a badly infected district in Africa, found no less than 16.6 per cent. of *Anopheles* caught in the huts of the natives to be infected, and Bentley, in Bombay, found 10 per cent. of *Anopheles stephensi* infected. At Camp Stotsenburg, the most malarial military post in the Philippine Islands, during the height of the malarial season, as high as 35 per cent. of the anopheline mosquitoes examined showed infection with the plasmodia, but at other times the percentage was small, sometimes not more than one per cent.

As already stated, the small percentage of infected mosquitoes noted in most malarial regions is sufficient to produce much malaria, because a single infected insect may transmit the plasmodia to many human hosts, as mosquitoes bite repeatedly and attack different individuals. Mitzmain (1916) was able to experimentally infect five individuals by the bites of two infected *Anopheles punctipennis* in one experiment, and four individuals by the bites of two *Anopheles punctipennis*, one of which has been used in the preceding experiment.

Another factor of great importance in the transmission of the malaria plasmodia to man is the *particular species* of *Anopheles* present in a locality. It has been proven by numerous observers that certain species of anopheline mosquitoes are much more efficient as hosts and transmitters of the malaria plasmodia than are others, and that some species do not act in nature as transmitting agents, although they may be experimentally infected. This fact was first demonstrated by Stephens and Christophers (1904) in India. They dissected 328 *Anopheles culicifacies*, and found



5.4 per cent. infected with *sporozoites* of the malaria plasmodia, while not a single *Anopheles rossi*, of the 870 dissected by them, showed infection with the plasmodia. Their observations showed that *A. rossi* was not an important transmitting agent of malaria in India, and although this species can be experimentally infected, it is not an active natural transmitter of the plasmodia.

The observations of Stephens and Christophers were confirmed by Bentley, in Bombay, who examined 837 *Anopheles stephensi* and found 10 per cent. showing oöcysts of the malaria plasmodia, and 3.5 per cent. showing *sporozoites* in the salivary glands, while the examinations of *A. rossi* were invariably negative.

Not only are some of the *Anophelinae* unable, under natural conditions, to act as hosts of the plasmodia, but some species can serve as an efficient host of certain species of plasmodia, while other species cannot develop within the insect. Thus it has been found in Japan that *Anopheles formosensis* and *Anopheles cohesus* act as efficient transmitters of the æstivo-autumnal subtertian plasmodium (*Plasmodium falciparum*), while *Anopheles jesoensis* acts as the transmitting agent of both the benign tertian and the subtertian æstivo-autumnal plasmodia, *Plasmodium vivax* and *Plasmodium falciparum*. Mitzmain (1917) has shown that in the United States *Anopheles quadrimaculatus* is an efficient host of *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium falciparum*, while *Anopheles punctipennis* and *Anopheles crucians* are efficient hosts of *Plasmodium vivax* and *Plasmodium falciparum*. In the Canal Zone, Darling (1900) experimented with *Anopheles albimanus*, *Anopheles pseudopunctipennis*, and *Anopheles malefactor* by allowing the insects to feed upon patients whose blood showed gametes of *Plasmodium vivax* and *Plasmodium falciparum*. Of the 100 mosquitoes examined, 70.8 per cent. of *Anopheles albimanus* became infected with plasmodia; 12.9 per cent. of *Anopheles pseudopunctipennis* became infected, and none of *Anopheles malefactor*. From these observations Darling concluded that the chief transmitting insect of malaria in the Canal Zone, Panama, was *Anopheles albimanus*, while *Anopheles pseudopunctipennis* occasionally transmitted the plasmodia, but that *Anopheles malefactor*, despite its significant name, never transmitted the plasmodia, and his conclusions have been repeatedly confirmed by others.

While it is probably possible to infect most species of *Anopheles* mosquitoes experimentally with the malaria plasmodia, it is true that a comparatively few of the very numerous species of *Anopheles* actually act in nature as transmitting agents of the plasmodia to man. The following table gives the species that are believed to be the transmitting agents of malaria in the regions mentioned, but some of the species act in this way to a very limited extent. Thus in India, *Anopheles rossi* is a very inefficient trans-



mitting agent of the plasmodia, and many authorities believe that this species never transmits the parasites of malaria. The same is true of other species given in the table, but they are included, as the evidence appears to be sufficient to prove that under favorable conditions they may transmit the plasmodia, and instances have been reported of their activity in this direction.

*Mosquitoes Concerned in the Transmission of the Malaria Plasmodia in Different Regions*

Region	Species of Anopheles
United States.	<i>A. quadrimaculatus</i> . <i>A. punctipennis</i> . <i>A. pseudopunctipennis</i> . <i>A. crucians</i> .
South America.	<i>A. albimanus</i> . <i>A. argyritarsus</i> . <i>A. cruzi</i> . <i>A. intermedius</i> . <i>A. punctipennis</i> . <i>A. pseudopunctipennis</i> . <i>A. pseudomaculipes</i> . <i>A. tarsimaculata</i> .
Panama.	<i>A. albimanus</i> . <i>A. argyritarsus</i> . <i>A. pseudopunctipennis</i> . <i>A. tarsimaculata</i> .
Europe.	<i>A. maculipennis</i> . <i>A. superpictus</i> . <i>A. sinensis</i> . <i>A. turkhudi</i> .
Asia.	<i>A. barbirostris</i> . <i>A. culicifacies</i> . <i>A. funestus</i> . <i>A. fuliginosus</i> . <i>A. ludlowi</i> . <i>A. maculatus</i> . <i>A. maculipalpis</i> . <i>A. minimus</i> . <i>A. rossi</i> . <i>A. stephensi</i> . <i>A. sinensis</i> . <i>A. theobaldi</i> . <i>A. umbrosus</i> .
Africa.	<i>A. turkhudi</i> . <i>A. willmori</i> . <i>A. culicifacies</i> . <i>A. funestus</i> . <i>A. costalis</i> . <i>A. maculipennis</i> . <i>A. maculipalpis</i> . <i>A. mauritanus</i> . <i>A. pharænsis</i> . <i>A. turkhudi</i> . <i>A. umbrosus</i> .
Philippine Islands.	<i>A. barbirostris</i> . <i>A. fuliginosus</i> . <i>A. ludlowi</i> . <i>A. maculatus</i> . <i>A. minimus</i> . <i>A. sinensis</i> .
Porto Rico.	<i>A. albimanus</i> . <i>A. argyritarsus</i> . <i>A. tarsimaculata</i> .

The above table does not include all anophelines that have been experimentally infected with the malaria plasmodia, but only those that are believed to be of importance in the natural transmission of the parasites.

*Humidity* is another factor of great importance in the transmission of the plasmodia by the mosquito. This factor acts by influencing the reproduction and activities of the insect, and probably, as stated by Christophers (1923), by affecting the internal temperature of the mosquito, thus influencing the development of the plasmodia within the insect. This subject deserves more extended study than has yet been given to it, and important developments may follow such studies.

Among other factors which influence the transmission of the malaria plasmodia to man by the mosquito may be mentioned the *habits* of different species of mosquitoes, the *character of the soil*, and the nature of the *economic conditions* of the population in each locality. The mosquitoes that are domesticated species are the most dangerous as transmitters of the plasmodia, as such species are brought most intimately in contact with man. Thus *Anopheles maculipennis* and *Anopheles quadrimaculatus*, the most efficient transmitters of the plasmodia in Europe and the United States,

respectively, are domestic in their habits, living in close proximity to the habitations of man, and frequenting such habitations, while species like *Anopheles bifurcatus* and *Anopheles umbrosus* are less active, owing to the fact that these species live in the open or in forests and are not brought so frequently into contact with man. The character of the soil influences the transmission of the plasmodia in so far as it favors the breeding of the transmitting mosquitoes by providing pools of water by the retention of surface supplies. The economic condition of the inhabitants of a region influences the transmission of the plasmodia by the mosquito indirectly. If poverty is present, with its attendant lack of food, the resistance of the people is lowered, and they are more susceptible to infection; if the industry of the region is one that requires out-of-door employment transmission is favored, owing to the facility with which the mosquito may reach the individual; while if the population depends for its agricultural products upon irrigation, the transmission of the plasmodia is greatly favored by the facilities afforded mosquitoes for breeding in the irrigation ditches.

The factors mentioned that influence the transmission of the malaria plasmodia by acting upon the insect host are by no means all that may be concerned, and it is very probable that we are yet ignorant of many factors that may have a profound influence in this direction, and may explain some of the divergent phenomena one observes in the study of the relationship of the mosquito to the transmission of the plasmodia.

**Factors Concerning the Recipient of the Plasmodia.**—It has already been mentioned that decreasing the natural resistance of the individual favors the transmitting of the malaria plasmodia by the mosquito, and this observation has led to a study of immunity as related to malarial infection. It may be stated that no race of people are naturally immune, as a race, to malaria, but a natural immunity to the infection does occur in the case of individuals, and there are well-authenticated cases on record of individuals living in intensely malarial regions who have never suffered from the infection. Celli (1900) instances the case of four individuals, living near Sezze, in the Pontine Marshes, a most malarious region, who have resided there for years, have never taken any precautions against malaria, yet who have never suffered from the disease. He says: "They work very laboriously, have insufficient and bad food, frequently sleep on the marshes, in the open air, and in such a manner as to be continually bitten by mosquitoes; still they never had malaria, are very healthy, have a rosy color, and their liver and spleen are normal in size." Marchiafava and Bignami (1909) note an interesting example of natural immunity in a family residing in one of the worst malarial districts of the Roman Campagna. The grandfather, the father, and two sons, although continually exposed to infected mosquitoes, had never had

malaria, although practically every one living in the same locality for any length of time developed the disease. I have observed several instances of natural immunity in officers and soldiers of the American troops in Cuba and the Philippines. These individuals, although serving in very malarial regions, and taking no precautions against infection, never suffered from malaria, although their comrades developed the disease.

While natural immunity to infection with the malaria plasmodia is not a general or racial characteristic, a relative acquired immunity is observed in many races living in malarial regions. Long residence in a malarial region, accompanied by repeated attacks of malaria, will confer a relative immunity to the plasmodia, and in such regions the examination of the blood of infants and adults show that frequently the plasmodia are present without any symptoms of the infection being noted. In such localities the presence of a relatively immune population hinders the transmission of the plasmodia by the mosquito, so far as clinical evidences of such transmission are concerned. This subject will be found more fully discussed in considering the prophylaxis of the malaria infections, but there can be no doubt that the relative immunity of certain populations has a very marked effect upon the transmission of the plasmodia, and this effect may be a favorable one, in that it has been found that the immunity present is not an immunity to the plasmodia *per se*, but to the toxins produced by the development of the plasmodia in the blood, so that infection is not prevented, but only the symptoms of the infection. It follows, therefore, that many individuals become "carriers" of the plasmodia without being aware of the fact, and that thus the infection of mosquitoes is favored, and the transmission of the plasmodia by the insects facilitated. In such regions the percentage of infected mosquitoes is often found very high, and practically all non-immune strangers coming into the region are infected and develop symptoms of malaria, although the native inhabitants appear clinically to be free from the infection. In such regions the appearance of non-immunes is always followed by an outbreak of malaria and, if the non-immunes are numerous enough, by an epidemic of the disease.

**Species of Plasmodia Occurring in Lower Animals.**—Several observers have described species of plasmodia occurring in lower animals, but, so far as known, none of these species can live in man. The human plasmodia have been found, according to some authorities, in the blood of the higher apes, as the chimpanzee, but further research is necessary before this interesting and important epidemiological question is settled. Among the species of plasmodia that have been described in the lower animals the most important are the following:

*Plasmodium bovis*, Kolle, 1898. A plasmodium observed in the blood

of cattle in South Africa, and said to cause remittent fever and severe and persistent anæmia in these animals.

*Plasmodium kochi*, Laveran, 1899. Observed in the blood of chimpanzees (*Anthropopithecus troglodytes*) and other monkeys in Ceylon and Africa.

*Plasmodium vassali*, Laveran, 1905. Found in the blood of a squirrel (*Sciurus griseimanus*) by Vassal.

*Plasmodium cynomolgi*, Mayer, 1907. In the blood of monkeys (*Macacus cynomolgus*). Resembles the tertian plasmodium of man, *Plasmodium vivax*.

*Plasmodium inui*, Halberstædter and Prowazek, 1907. A parasite of the blood of monkeys (*Macacus cynomolgus* and *Macacus nemestrinus*). This species resembles *Plasmodium vivax*.

*Plasmodium pitheci*, Halberstædter and Prowazek, 1907. Found in the blood of the ourang-outang (*Simia satyrus*) and the chimpanzee (*Anthropopithecus troglodytes*). This plasmodium resembles both *Plasmodium vivax* and *Plasmodium malariae*.

*Plasmodium canis*, Castellani and Chalmers, 1910. This plasmodium was found to be a common parasite in dogs in Ceylon by Castellani and Chalmers. It resembles very closely in its morphology the tertian plasmodium of man, *Plasmodium vivax*.

*Plasmodium equi*, Castellani and Chalmers, 1913. This parasite was found by these authors in the blood of a horse in Ceylon. It resembles *Plasmodium canis*.

*Plasmodium richenowi*, Blacklock and Adler, 1924. This plasmodium was found in the blood of chimpanzees and gorillas by Richenow, and in chimpanzees by Blacklock and Adler. It resembles *Plasmodium falciparum*, the tertian æstivo-autumnal parasite of man.

*Plasmodium danilewski*, Grassi and Feletti, 1890. This parasite of birds really belongs to the genus *Proteosoma*, but is included by some zoologists in the genus *Plasmodium*. It is the plasmodium with which Ross first worked out a developmental cycle in the mosquito, the organism undergoing its asexual cycle of existence in the blood corpuscles of sparrows, and its sexual cycle of existence in mosquitoes belonging to the genus *Culex*.

Among other plasmodia that have been described may be mentioned *Plasmodium brasilianum*, Gonder and Gossler, 1908, in *Brachyurus calvus*; *Plasmodium monosoma*, Vassal, 1907, in *Vesperugo abramus*; *Plasmodium murinum*, Dionisi, 1898, in *Myotis myotis*; *Plasmodium vauhani*, Novy and MacNeal, in *Merula migratoria*; *Plasmodium diploglossi*, Aragão and Neiva, 1900, in *Diploglossus fasciatus*; and *Plasmodium tropiduri*, Aragão and Neiva, 1909, in *Tropidurus torquatus*. It is more than probable that further research will demonstrate that some of these



plasmodia are not distinct species, or that they belong to some other genus, as the descriptions are often insufficient and confusing and, in some of the plasmodia, of little scientific value.

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## CHAPTER XIV

THE MALARIA PLASMODIA (CONTINUED). *PLASMODIUM VIVAX*.  
*PLASMODIUM VIVAX MINUTUM*. *PLASMODIUM MALARIÆ*.

In describing the malaria plasmodia in detail the following classification will be followed:

**Genus.**—*PLASMODIUM*, Marchiafava and Celli, 1885.

**Species I.**—*PLASMODIUM VIVAX*, Grassi and Feletti, 1890.

**Subspecies I.**—*PLASMODIUM VIVAX MINUTUM*, Emin, 1914.

**Species II.**—*PLASMODIUM MALARIÆ*, Laveran, 1881.

**Species III.**—*PLASMODIUM FALCIPARUM*, Welch, 1897.

**Subspecies II.**—*PLASMODIUM FALCIPARUM QUOTIDIANUM*, Craig, 1909.

**Doubtful Species I.**—*PLASMODIUM TENUE*, Stephens, 1914.

**II.**—*PLASMODIUM OVALE*, Stephens, 1922.

**Species I.** *PLASMODIUM VIVAX*, Grassi and Feletti, 1890.

Synonyms: *Oscillaria malarie*, Laveran, 1881, *pro parte*. *Plasmodium*, var. *tertiana*, Golgi, 1889. *Hæmamæba vivax*, Grassi and Feletti, 1890. *Hæmamæba malarie*, var. *magna*, Laveran, 1890. *Hæmamæba laverani*, var. *tertiana*, Labbé, 1894. *Hæmosporidium tertianum*, Lewkowitz, 1897. *Plasmodium malarie tertianum*, Labbé, 1899. *Hæmamæba malarie*, var. *tertiana*, Laveran, 1901. *Plasmodium tertiane*, Billet, 1904, *pro parte*.

**History and Nomenclature.**—*Plasmodium vivax*, the cause of tertian malaria, was probably first observed by Laveran, in 1880, as some of the intracellular pigmented bodies described by him may be interpreted as stages in the life-cycle of this plasmodium, but it was not recognized by him as a distinct species. Golgi, in 1886, was the first to describe this plasmodium as a distinct species and differentiated it from *Plasmodium malarie*, the quartan parasite. He noted that sporulation in *Plasmodium vivax* occurred every 48 hours instead of every 72 hours, as in *Plasmodium malarie*, and that the paroxysm of chill, fever, and sweating occurred at the time of sporulation. He described the morphological features that distinguish the plasmodium from other malaria plasmodia, and stated that a quotidian fever might be caused by a double infection with this parasite, each group sporulating upon successive days. Although Golgi considered it as a distinct species he did not give it a specific name, and the name, *Plasmodium vivax*, was given the plasmodium in 1890 by Grassi and Feletti.

**Morphology.**—As already stated, the malaria plasmodia present forms in the blood of man that undergo their development entirely within the red blood corpuscles and others that are intended to undergo their development in the mosquito. In describing the morphology of the plasmodia it

is thus necessary to describe that of the forms concerned in *schizogony*, or the human life-cycle, and the forms known as *gametes* which are developed in the blood, and which, when they reach the stomach of the mosquito, initiate the mosquito cycle of existence, or sporogony. As it is important that one be able to recognize these parasites in both fresh unstained preparations of blood and stained preparations, the morphology of the plasmodia as observed in such preparations will be described separately.

**Morphology of Plasmodium vivax in Unstained Preparations.**—*Plasmodium vivax*, or the tertian plasmodium, completes its development in the blood of man in 48 hours, giving rise to the well-known type of malarial fever, associated with a chill and fever occurring upon every second day. The parasite appears first upon or within the red blood corpuscle as a small, amœboid hyaline disk or "ring," the *trophozoite*, measuring about 2 microns in diameter; its outline is very indistinct, and in many instances the organism at this stage of development is overlooked by reason of the absence of pigment and its delicate, veil-like appearance. As the parasite grows older it develops very marked amœboid activity, constantly changing its shape, but is still indistinct in outline, requiring very careful focussing in order to distinguish it in the red corpuscle, and it is not until pigment develops that it can be easily recognized.

In the course of from six to eight hours minute granules of a reddish-brown pigment may be observed within the hyaline cytoplasm, and the outline of the parasite becomes more clearly distinguishable. This is the earliest stage in the development of the *schizont*. The pigment is of a peculiar reddish-brown color and in the form of very fine granules distributed irregularly throughout the cytoplasm and possesses very active dancing motility, apparently due to currents within the cytoplasm. As development proceeds amœboid motion becomes less pronounced, and when the parasite is fully grown no amœboid motion can be detected. The pigment, at first small in amount, increases gradually as the parasite enlarges, and is actively motile until a short time before sporulation, at which time it collects in larger masses or in a single irregular mass in the cytoplasm.

At the end of 24 hours the plasmodium fills more than half of the infected red corpuscle, which is larger than normal in size and lighter green in color. The parasite contains much actively motile pigment and varies greatly in shape, due to its very marked amœboid motility. Very often at this stage of development multiple infection of the red corpuscle may be suspected by reason of the appearance of two or more spherical bodies containing pigment in the same cell, but careful examination will reveal the fact that the pigmented spherical bodies are but portions of the amœboid pseudopodia of a single organism, the remainder of the parasite being situated deeper within the red cell, and thus rendered invisible for the time



being. However, multiple infection of the red corpuscle with two plasmodia is not infrequently observed.

At the end of 36 hours the plasmodium has attained almost full growth, and practically fills the red blood corpuscle.

Amœboid motility has practically disappeared, or is very sluggish, but the pigment continues to be actively motile, has increased in amount, and is distributed in the form of fine granules throughout the cytoplasm; the outline of the plasmodium is well defined, contrasting well with the light-green border of the red corpuscle which surrounds it. The infected red blood corpuscle is almost twice as large as the uninfected corpuscles and is much lighter green in color. When fully grown *Plasmodium vivax* measures from 9 to 11 microns in diameter, but larger forms are often observed.

At the end of 48 hours sporulation or segmentation occurs. The pigment becomes collected at the centre or to one side of the plasmodium in the form of a compact clump, dark brown in color, and fine radial striations are observed in the parasite extending from the centre toward the periphery, dividing it into several ovoid segments or spores, the *merozoites*. As a rule the *merozoites* are arranged in two rows, one row surrounding the pigment at the centre of the plasmodium and another surrounding the first row, but very often the *merozoites* are arranged irregularly. The *merozoites* are always devoid of pigment, measure from 1.5 to 2 microns in diameter, and number from 12 to 24, the average number being 16 to 20; they are oval in shape, hyaline in appearance, and have a spherical refractive centre. The *merozoites* are capable of attaching themselves to the red blood corpuscle and penetrating it, and this occurs as soon as they are liberated into the blood plasma by the rupture of the host cells at the time of sporulation. Sporulation covers several hours, so that, while it is stated that it occurs at the end of 48 hours, as a matter of fact it begins when the plasmodia are about 42 hours old, from 5 to 6 hours being covered by the process. At the time of sporulation the infected red blood corpuscle consists of a narrow rim of cytoplasm surrounding the sporulating parasite, and when the process is complete there is no evidence of the infected corpuscle except a small amount of granular débris in the vicinity of the liberated *merozoites*.

As soon as the *merozoite* reaches its host cell, the red blood corpuscle, it becomes a *trophozoite*, and the process of *schizogony* is repeated. After several generations of plasmodia have thus reproduced, there are developed in the blood of man forms of the parasite intended to undergo their further development in the mosquito. These forms are called *gametes* and are sexual in nature, the male being called a *microgametocyte* and the female a *macrogametocyte*. These bodies do not sporulate, but become free in the blood plasma as spherical bodies which may be easily differentiated from

the sporulating plasmodia. In the earliest stage of development within the red blood corpuscle the *gametes* are never "ring" shaped, but appear

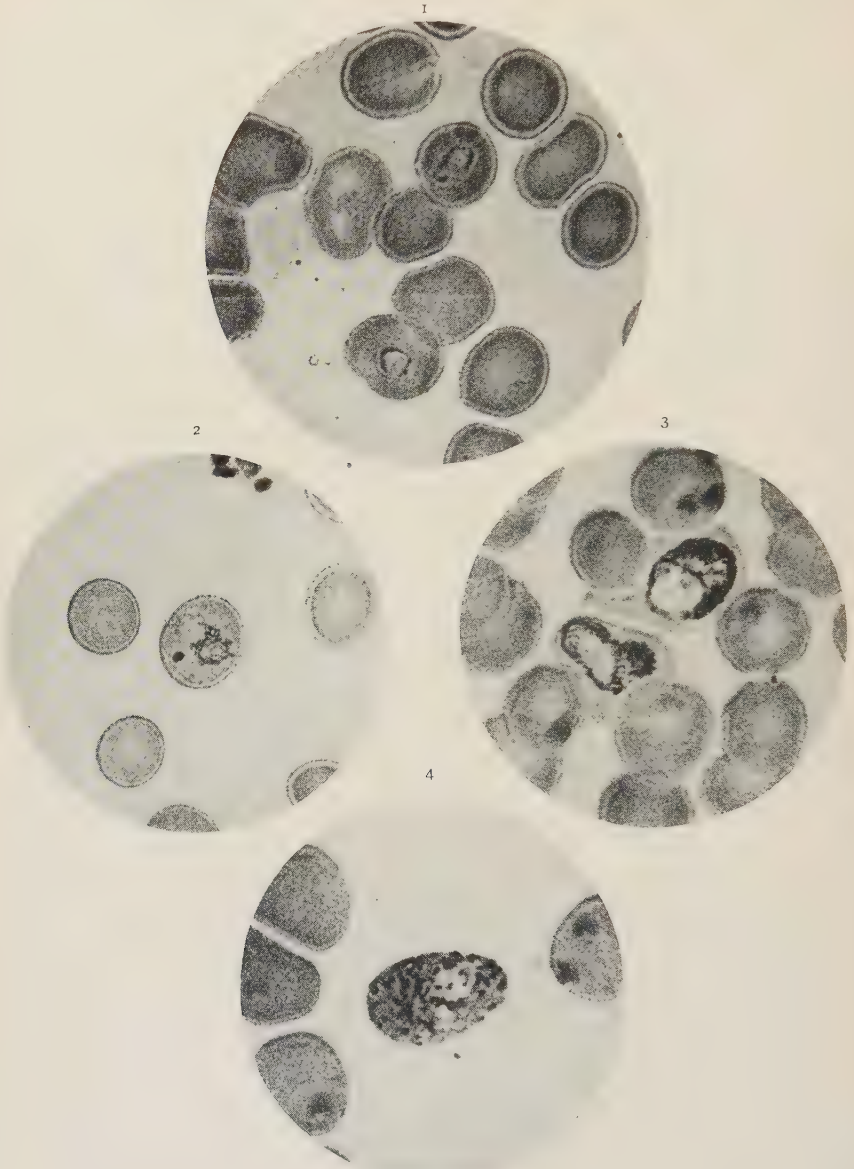


FIG. 68.—*Plasmodium vivax*. (Photomicrographs. Army Medical School Collection.) Stained with Wright's stain. 1. Young trophozoites, or "ring-forms."  $\times 1,200$ . 2. Quarter grown schizont.  $\times 1,200$ . 3. Half-grown schizont.  $\times 1,500$ . 4. Three-quarters grown schizont.  $\times 1,800$ .

as oval or spherical solid hyaline bodies devoid of pigment. Development is much slower than in the case of the *schizonts*, but as growth occurs pig-

ment develops, but in larger amount and in coarser granules than in the *schizont*. Amœboid motility is much less marked, and ceases entirely when the *gamete* is about half grown. The infected red blood corpuscle is enlarged and anæmic in appearance, and when fully developed the *gamete* entirely fills the cell.

In unstained preparations the *microgametocytes* and *macrogametocytes* can be easily distinguished when fully developed. The *microgametocyte* measures from 8 to 10 microns in diameter, is spherical in shape, and contains a large amount of pigment in the form of large and small granules uniformly distributed throughout the cytoplasm. A hyaline oval area is situated somewhere within the parasite, which is devoid of pigment and which represents the vesicular nucleus of the organism. When the *microgametocyte* is first liberated from the red corpuscle in which it has developed, the pigment is sluggishly motile, but in those *microgametocytes* in which *microgametes* are developing the pigment soon becomes very actively motile, much more so than at any stage of development of the *schizont*, while the cytoplasm of the parasite also appears to be in motion, marked undulations of the periphery being visible. If such an organism be watched for a variable period of time—from five minutes to half an hour or more—the pigment will be seen to collect toward the centre of the body, the motility being lessened, and suddenly, as though an explosion had occurred within the parasite, there will appear at certain portions of the periphery long, thin, colorless, thread-like, actively moving filaments, which vary in number from two to six, and which undulate rapidly, lashing about among the red blood corpuscles, to which they often impart a peculiar spinning motion. These filaments are the *microgametes*, and, under natural conditions, their development occurs within the stomach of the mosquito, but the process may readily be observed in blood taken from the finger upon a slide which has been previously moistened by breathing upon it.

After the extrusion of the *microgametes* by the *microgametocytes* they become free in the blood plasma, and eventually degenerate. The *microgametes* are never produced in the blood of man, but only when the blood has been removed from the body and under conditions which apparently simulate the conditions present in the stomach of the mosquito. As observed in unstained preparations of blood, the *microgamete* of *Plasmodium vivax* is a very slender, thread-like body, hyaline in appearance, and having a serpentine, undulating motion which enables it to progress among the red blood corpuscles, which are displaced or moved about by its motions. The extremities are pointed and very hard to distinguish, and small nodular swellings are sometimes noted along the body, which may contain minute granules of pigment derived from the *microgametocyte*. They vary greatly in length, some being several times as long as the diameter of the *microgametocyte*, while others are much shorter. Before fertilizing the

*macrogamete* the *microgamete* extrudes any pigment which may have been present, and is perfectly hyaline in appearance.

The *macrogametocyte* of *Plasmodium vivax*, when unstained, appears as a large pigmented, oval or spherical body, measuring from 11 to 15 microns in diameter; the cytoplasm is finely granular and the pigment is in the form of large granules or clumps, collected toward the periphery of the parasite or arranged in a wreath-like form at a little distance from the periphery. The pigment is not motile, and is of a dark-brown color. The wreath-like arrangement of the pigment does not take place until maturation phenomena are completed, at which time the organism is known as a *macrogamete*, and is ready for fertilization by the *microgamete*. If blood containing both *macrogametocytes* and *microgametocytes* be collected upon a moistened microscopic slide and examined, it will be noted that while exflagellation never occurs in the *macrogametocytes*, in rare instances a flagellum may be seen attached to a *macrogamete*, and such an appearance always indicates an attempt at fertilization. Sometimes more than one flagellum or *microgamete* may be observed attached to a *macrogamete*, and their movements are peculiar and characteristic. Instead of the rapid serpentine lashings noted when the *microgametes* are attached to a *microgametocyte*, they appear to straighten out and then relax, at the same time revolving very rapidly; sometimes they may be seen to pull themselves loose from the *macrogamete* and again become attached to it. The pigment maintains its wreath-like arrangement within the organism, and is at most very slightly motile. The movements observed are produced by the efforts of the *microgamete* to penetrate the *macrogamete*, a process which normally occurs within the stomach of the mosquito, but which may rarely be observed in preparations of blood under the microscope.

**Morphology of *Plasmodium vivax* in Stained Preparations.**—The morphology of *Plasmodium vivax* in preparations of blood stained by the Romanowsky stain, or any of its modifications, is characteristic, and the staining reactions of the infected red blood corpuscles still more so. The stain that I have found most useful for the study of the malaria plasmodia is the Wright stain, and the following description of the morphology of *Plasmodium vivax* is based upon preparations stained by this method.

*Historical Summary.* The first observations relating to the structure of the malaria plasmodia as shown in stained preparations of blood were those of Celli and Guarnieri, who used methylene blue as the staining reagent. They described a deeply stained ectoplasm and a dimly stained endoplasm, the latter forming the centre of the plasmodium. In the "ring-forms" they described a deeply stained dot situated between the ecto- and endoplasm, which later observers demonstrated to be the chromatin of the nucleus. Grassi and Feletti considered that the dimly stained endoplasm was in reality a large vesicular nucleus, but to Romanowsky



we owe our exact knowledge of the structure of the plasmodia. Using his stain, a mixture of methylene blue and eosin, he demonstrated that the plasmodia consist of an outer, deeply stained portion, the cytoplasm, and an inner, unstained portion, the nucleus. The latter is spherical or oval in shape and presents at some portion of its periphery a deeply stained mass, the chromatin of the nucleus. This description applies only to the young plasmodia, as in the older organisms he found that the large vesicular nucleus disappeared, and that the chromatin became distributed in the cytoplasm of the plasmodia.

*Morphology.* The youngest form of plasmodium seen in stained preparations of blood is a small oval or spherical ring-like body upon the red blood corpuscle or within it. Very often these little "rings" may be seen attached to the corpuscle and projecting from its periphery. The ring-like appearance is due to the presence of a large vacuole within the parasite which apparently forms a part of the infected red corpuscle, as the color of the latter shows through it. At this early stage of development the parasite consists of a deep-blue ring of cytoplasm, lying in the orange or pink stained red corpuscle, and having at one portion of the periphery of the ring a deep-red dot of chromatin, which indicates the position of the nucleus. The chromatin varies in the intensity with which it takes the stain, and may be a ruby red, violet, or almost black color. It is generally surrounded by a milky white, spherical or oval, area, which represents the unstained portion of the vesicular nucleus, and this area is in contact with the unstained vacuole comprising the centre of the "ring," which is apparently stained in the same way as the red corpuscle. The blue ring of cytoplasm may be very delicate and of the same breadth throughout, but it is generally thicker at one portion, giving the parasite the so-called "signet-ring" appearance. The young ring forms usually measure from 2.5 to 3 microns in diameter, but some may be larger, and "rings" measuring as much as 4 to 5 microns in diameter have been observed.

As the organism develops and amœboid motility becomes pronounced the "ring-forms" become distorted, due to minute pseudopodia that arise from the cytoplasm. In such instances parasites are observed in which delicate filaments of blue-stained cytoplasm may be seen connecting various portions of the periphery of the "ring" or projected from the periphery, and the chromatin dot may be situated at any portion of the pseudopodia, or at the point of attachment of a pseudopodium to the body of the plasmodium. In the youngest "ring-forms" it is not very uncommon to observe two dots of chromatin, either situated near one another or at different portions of the cytoplasm.

As the parasite becomes larger and pigment begins to develop, the amœboid activity of the plasmodium increases and stained preparations present many bizarre forms of the plasmodia which it is impossible to

describe. The cytoplasm stains blue, and in it is distributed the pigment, which stains a greenish-black color. At the beginning of pigmentation both the large vacuole and the vesicular nucleus may be distinguished, but both disappear with the growth of the parasite, and the nuclear chromatin, which has increased in amount, is distributed in the cytoplasm, where it appears as minute granules and filaments stained a pale pink. At a certain period of development the chromatin divides into very fine grains and filaments, and only prolonged staining will demonstrate its presence.

As the *schizont* approaches maturity the cytoplasm, which has increased greatly in amount, stains a deep blue and is heavily pigmented. The chromatin stains an intense ruby red or violet, and is distributed in the cytoplasm in irregular clumps, the filamentous structure having disappeared. The vesicular portion of the nucleus and the large vacuole are no longer visible.

In the presporulating plasmodia the cytoplasm, containing the pigment, stains a well-defined blue, the pigment being arranged in irregular masses staining a peculiar greenish brown. The chromatin, staining an intense red or violet, is collected in roughly spherical masses, arranged more or less regularly in the cytoplasm, which shows no evidence of division.

In the segmenting, or sporulating, plasmodia the chromatin is collected in compact, deep-red or violet spherical masses, each surrounded by an unstained area, in turn surrounded by a rim of blue-stained cytoplasm, and the pigment is collected into a single mass near or at the centre of the organism, or in one or two irregular masses situated in a small portion of residual cytoplasm. When the infected erythrocyte disintegrates the spores, or *merozoites*, appear free in the preparation and are seen to consist of a round or oval mass of cytoplasm, stained a deep blue, enclosing an unstained oval or round area which contains a spherical mass of chromatin stained a deep red or violet. The chromatin is generally placed eccentrically, and the cytoplasm is thicker at that portion of the parasite furthest removed from the chromatin. The *merozoites*, in stained preparations, measure from 1.5 to 2 microns in diameter.

The description of *Plasmodium vivax* which has been given refers only to the forms concerned in the human life-cycle of the parasite, but certain forms are developed during this cycle that undergo development in the mosquito, the *gametes*, and these differ in their morphology from the *schizonts*, and will now be described.

*Morphology of Gametes.* The *gametes* of *Plasmodium vivax* in their earliest stage of development, when stained by Wright's stain, consist of a circular mass of blue-stained cytoplasm, in the centre of which is a spherical dot of chromatin stained a deep red or violet. The unstained zone, or nutrient vacuole, which gives the "ring" appearance to the young *schizont*, is not present, and the parasite at this stage of develop-

ment is slightly larger than the *schizont*. Later, when pigment develops, it is larger in amount than in the *schizonts*, and is distributed throughout the cytoplasm. The *gametes* are generally circular or oval in shape, and the bizarre forms, so characteristic a feature of the *schizonts*, are not observed. The *microgametocytes* (male) and the *macrogametocytes* (female) can be readily differentiated in stained preparations.

The *microgametocyte*, or male *gamete*, stains much less intensely than the *macrogametocyte*, or female *gamete*, the cytoplasm at every stage of growth staining pale blue, and the nucleus is large, consisting of granules of chromatin arranged loosely in minute masses, the entire nucleus, in the fully developed forms, resembling a spindle lying within the cytoplasm. In the youngest forms the nucleus is represented by a single round mass of chromatin. In those *microgametocytes* which are about to flagellate the chromatin may be seen divided into four to eight masses which are collected near the periphery of the parasite, and such forms may be mistaken for presporulating parasites. *Microgametocytes* which are flagellating often show a thread of deep-red chromatin extending into the flagella. The pigment in the *microgametocytes* is large in amount and distributed throughout the cytoplasm in the form of minute dots or slender, short rods, which stain a greenish black.

The *chromatin* of the nucleus is much larger in amount in the *microgametocytes* than in the *macrogametocytes*, and is often collected within the nucleus in large irregular masses or in well-defined fibrils which are of considerable thickness. It stains a deep red or violet, and is never distributed throughout the cytoplasm, but is confined to the nuclear area, at every stage of growth, except at the time of exflagellation. According to Ziemann, the proportion of chromatin to cytoplasm in the *microgametocytes* is from 1 to 1 to 1 to 4, while in the *macrogametocytes* the proportion is from 1 to 8 to 1 to 12. The *microgametocytes* are smaller than the *macrogametocytes* when fully developed, measuring 7 to 9 microns in diameter, while the latter measure from 10 to 14 microns in diameter.

The following points are useful in differentiating the *microgametocytes* of *Plasmodium vivax* from the *macrogametocytes*:

1. The pale-blue staining of the cytoplasm. Sometimes the cytoplasm is greenish in color.
2. The large amount of chromatin, its arrangement in masses or fibrils, and its loose arrangement in the nucleus.
3. The greater amount of pigment.
4. The presence of flagella in some preparations.
5. The smaller size of this form of the plasmodium.

The *microgametes* consist very largely of chromatin stained a red or violet, with a small amount of cytoplasm stained a light-blue color. When

attached to the *microgametocyte* the *microgametes* often appear to consist entirely of a delicate thread of violet chromatin, but when seen free in the

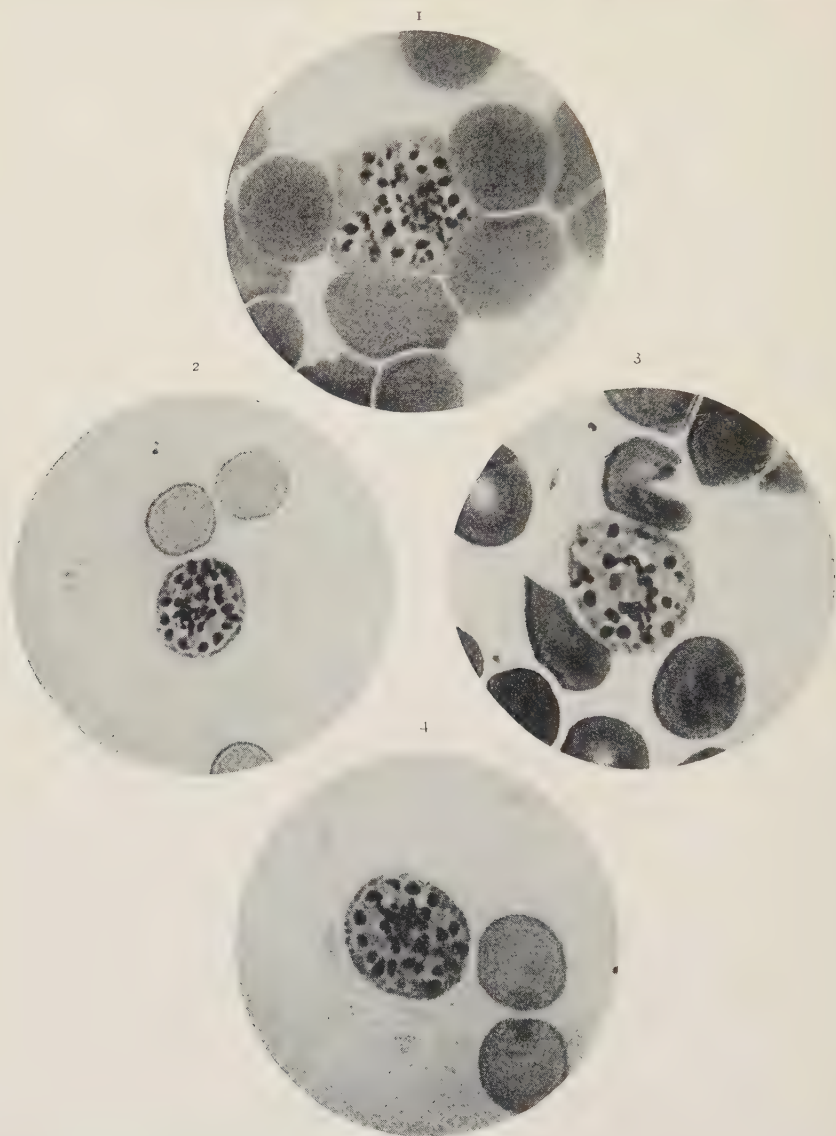


FIG. 69.—*Plasmodium vivax*. (Photomicrographs. Army Medical School Collection.) Stained with Wright's stain. 1. Presporulating schizont.  $\times 1,800$ . 2. Sporulating schizont.  $\times 1,200$ . 3. Sporulating schizont.  $\times 1,800$ . 4. Sporulating schizont.  $\times 1,800$ . Note enormous enlargement of the infected red blood corpuscle which has practically disappeared except for a narrow rim surrounding the parasites.

preparation a small amount of cytoplasm is generally visible. When free the *microgamete*, in stained preparations, has pointed ends and is slightly thicker at the middle than toward the extremities. The chromatin may be



divided into granules or irregular masses, but I believe that this appearance indicates degeneration.

The *macrogametocyte* of *Plasmodium vivax* is larger, in stained preparations, than the *microgametocyte*, and the cytoplasm stains more intensely, taking a deep-blue color with the Wright stain. The nucleus is much smaller, the nuclear chromatin being arranged in a compact mass near the periphery of the parasite. Even in the earliest stage of development the dot of chromatin representing the nucleus is smaller and more delicate in the *macrogametocyte* than in the *microgametocyte*. The pigment, while smaller in amount, stains a deeper black color, and is in the form of rods, which are arranged in minute masses or in a wreath-like arrangement toward the periphery of the organism. The *macrogametocytes* are always circular in contour, and may be distinguished from the *microgametocytes* by attention to the following points:

1. The deep-blue staining of the cytoplasm at all stages of development.
2. The smaller amount of chromatin and its compact arrangement within the nucleus.
3. The smaller amount of pigment.
4. The larger size of the *macrogametocyte*.

As the recognition of *gametes* of the malaria plasmodia is of great importance in the prophylaxis of malaria the following important differential features which distinguish them from the *schizonts* of *Plasmodium vivax* will be found useful:

1. The *gamete* is larger throughout its development than the *schizont*.
2. It is much less amœboid and is, therefore, circular or oval in contour in every stage of development within the erythrocyte.
3. No nutritive vacuole, or unstained area surrounding the chromatin of the nucleus, is present in the *gametes*. Thus, in the youngest forms, the "ring-like" appearance characteristic of the youngest *schizont* is not observed.
4. The pigment in the *gametes* is larger in amount and earlier developed than in the *schizonts*.
5. The chromatin of the *gamete* is never distributed throughout the cytoplasm, as it is at certain stages of development of the *schizont*, but is always collected in a restricted area.
6. The period of development within the red blood corpuscle is almost twice as long for the *gamete* as for the *schizont*.

When fully developed both the *microgametocyte* and *macrogametocyte* entirely fill the infected red blood corpuscle, which shows the same changes in staining reactions as the corpuscle infected by the *schizont*.

In stained preparations the relative number of male and female *gametes* can be easily ascertained, and it will be found that the number of each

varies in different cases. As a rule the female *gametes* are most numerous, but instances are frequent in which the male *gametes* are most numerous,

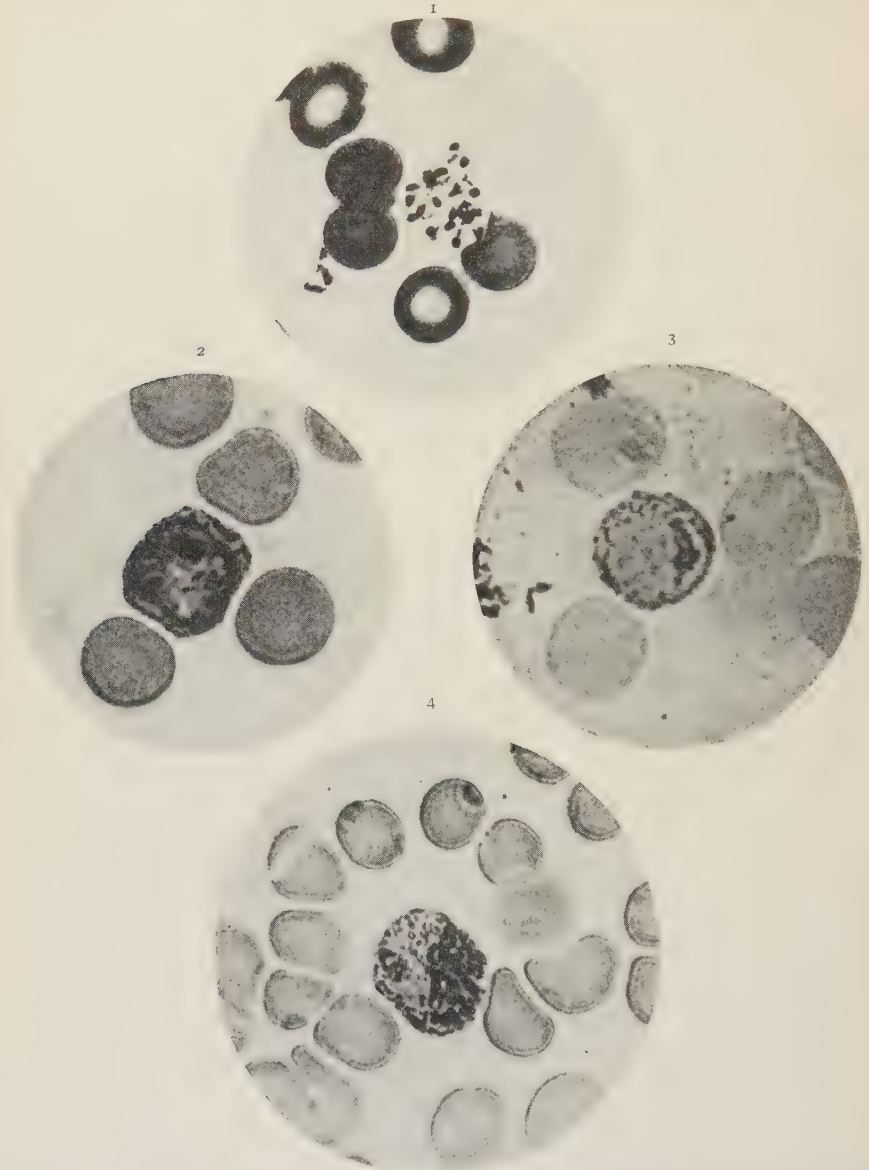


FIG. 70.—*Plasmodium vivax*. (Tertian malaria plasmodium.) (Photomicrographs. Army Medical School Collection.) Stained with Wright's stain. 1. Free spores or merozoites.  $\times 1,200$ . 2. Fully developed macrogametocyte.  $\times 1,800$ . 3. Fully developed microgametocyte.  $\times 1,800$ . 4. Atypical plasmodium, resembling the parthenogenetic macrogametes described by Schaudinn.  $\times 1,500$ .

while in other instances the number of male and female are about equal. It is very probable that the relative proportion varies somewhat in every

instance of infection due to conditions favoring the development of one or the other, brought about by the reaction of the system to the infection.

**Changes in the Infected Red Blood Corpuscles.**—The changes which occur in the red blood corpuscle infected with *Plasmodium vivax* are of great importance from a diagnostic standpoint, as these changes alone will suffice to differentiate this plasmodium from other malaria plasmodia occurring in man.

The red blood corpuscle infected by *Plasmodium vivax* is always larger than normal, even when the plasmodium is in the very early stages of development. Not only is the corpuscle enlarged but, in unstained preparations of blood, it appears paler in color, and in examining fresh specimens of blood for the plasmodium it is often most easily discovered by selecting any red corpuscles that appear larger and paler than normal, and carefully examining them. While the enlargement is not marked before the plasmodium becomes pigmented, the red cell enlarges rapidly after pigmentation occurs, and when the plasmodium is fully developed it is generally twice as large as the uninfected corpuscles and almost devoid of color. In stained preparations the enlargement is very noticeable, and the corpuscle is often much distorted, being oval or irregular in shape.

A very important degenerative change occurs in the cytoplasm of the red blood corpuscles infected by *Plasmodium vivax* which leads to the occurrence of small refractive granules within the cytoplasm, which stain a pink or light red when the Wright or other modifications of the Romanowsky stain is used. With the Wright stain this pink or red stippling of the infected corpuscle is well marked, and such cells are known as "stippled cells," and the granules as Schüffner's granules or dots. This appearance was first described by Schüffner, in 1899, and occurs only in the red blood corpuscles infected with *Plasmodium vivax* or *Plasmodium vivax*, var. *minutum*.

Schüffner's dots may not be present during the "ring" stage of the plasmodium, but as soon as pigment begins to develop these dots will be observed in stained preparations. In some infections with this plasmodium Schüffner's dots are not observed in the infected corpuscles, and the reason for this has not been ascertained. I have observed many cases of infection with plasmodia indistinguishable from *Plasmodium vivax*, in which the infected blood corpuscles never showed Schüffner's dots, while at the same time and place other infections were occurring in which every infected corpuscle showed this form of degeneration. The reports of infections with *Plasmodium malarie*, the quartan plasmodium, in which Schüffner's dots were observed in the infected red corpuscles, are explained, I believe, by the fact that in infections with *Plasmodium vivax*, var. *minutum*, a plasmodium much resembling *Plasmodium malarie* in

morphology, Schüffner's dots are sometimes observed in the infected red cell, and it is probable that this plasmodium was mistaken for *Plasmodium malarie*.

From a diagnostic standpoint the occurrence of Schüffner's dots in a red blood corpuscle containing a malaria plasmodium is positive evidence that the plasmodium is either *Plasmodium vivax* or *Plasmodium vivax*, var. *minutum*, and, as the latter species is very rare, the occurrence of these dots is practically diagnostic of *Plasmodium vivax*.

The characteristic changes occurring in the red blood corpuscle infected with *Plasmodium vivax* are the following: enlargement of the corpuscle; decolorization progressive with the growth of the plasmodium; changes in shape; and the occurrence of Schüffner's dots in the cytoplasm. Attention to these points will serve to differentiate infection with *Plasmodium vivax* from infections with the other malaria plasmodia.

**Habitat.**—*Plasmodium vivax* is a parasite of the red blood corpuscles of man, and efforts to inoculate it in other animals have invariably resulted negatively, although many observers have endeavored to transmit the plasmodium to the lower animals. There is no evidence of real scientific value that *Plasmodium vivax* ever occurs naturally in any of the lower animals, although plasmodia very similar in morphology have been found in the blood of various species of monkeys and apes by several observers, and some of these plasmodia are said to be indistinguishable in morphology from *Plasmodium vivax*.

**Species Occurring in Lower Animals.**—Several species of plasmodia resembling *Plasmodium vivax* in morphology have been described in monkeys and apes. Laveran, in 1899, described a plasmodium in the blood of chimpanzees in Ceylon and Africa which resembled this species. *Plasmodium cynomolgi*, Mayer, 1907, *Plasmodium inui*, Halberstædter and Prowazek, 1907, and *Plasmodium pitheci*, Halberstædter and Prowazek, 1907, are all species resembling in morphology *Plasmodium vivax* and found in the blood of monkeys and apes, the first two mentioned being found in monkeys belonging to the genus *Macacus*, and the last in the blood of the ourang-outang and the chimpanzee. In 1905, Vassal found a plasmodium in the blood of a squirrel (*Sciurus griseimanus*) which resembled *Plasmodium vivax* in morphology, and which Laveran named *Plasmodium vassali*, and in 1910, Castellani and Chalmers described a species resembling *Plasmodium vivax* in the blood of dogs in Ceylon, which they named *Plasmodium canis*. At present there is no evidence that any of these species are identical with *Plasmodium vivax*.

Donovan (1921) examined the blood of 86 monkeys in India for plasmodia, 76 *Macacus sinicus* and 10 *Presbitis priancus*, common species in that country, and found only one infection with a plasmodium



resembling *Plasmodium vivax* in morphology. However, in the blood of squirrels he found very frequently a plasmodium, which he named *Plasmodium ratufa*, that closely resembled *Plasmodium vivax* in morphology, but which is probably identical with *Plasmodium vassali*, Laveran, 1910, which Vassal found in the blood of the squirrel.

Richenow (1920) examined the blood of gorillas and chimpanzees in the Cameroons, and of eight chimpanzees one was found infected with a plasmodium identical in morphology with *Plasmodium vivax*, one with plasmodia resembling *Plasmodium vivax* and *Plasmodium falciparum*, and two with plasmodia resembling *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium falciparum*. Blacklock and Adler (1922) found a plasmodium resembling *Plasmodium vivax* in the blood of a chimpanzee, but forms also occurred resembling *Plasmodium falciparum* and *Plasmodium malariae*, and the only form of gamete found was a crescentic gamete resembling that of *Plasmodium falciparum*. From his observations Richenow believes that these animals may be important sources of infection of man with the malaria plasmodia, but the question of the identity of these plasmodia with *Plasmodium vivax* is still undecided, and the important question as to whether any animal acts as a reservoir of infection for man is unsolved.

**Cultivation.**—*Plasmodium vivax* was first cultivated *in vitro* by Bass (1911), using as a medium human blood to which a small percentage of dextrose was added. (See Chapter XIII.) His observations were soon confirmed by others, and it is now well established that it is possible to cultivate from two to as many as five generations of this plasmodium.

**Life-history.**—The life-history of *Plasmodium vivax* is similar to that of the other malaria plasmodia, and has been considered already in this work. (See Chapter XIII.) The development in the blood of man consumes forty-eight hours, at the end of which time the intracellular *schizont* divides into from 12 to 24 daughter cells, or *merozoites*, which infect new red blood corpuscles and repeat the human life-cycle, or *schizogony*. Certain forms, known as *gametes*, develop in the blood, which are ingested by anopheline mosquitoes, and which develop into *sporozoites* in these insects and infect man when the insect bites. The life-cycle in the mosquito consumes from 10 to 14 days under favorable conditions of temperature and moisture.

**Geographical Distribution.**—Infections with *Plasmodium vivax* are world-wide in distribution, and it is probable that this species of plasmodium is more widely distributed than are any of the other species of human malaria plasmodia. It is especially prevalent in temperate and semi-tropical regions, and in endemic malarial regions in the North Temperate Zone it is practically the only species constantly present. In

the tropics, although present wherever malaria is present, it is not so common a species as is *Plasmodium falciparum*, in most localities, although it may be the prevailing species in one locality and almost absent in another a few miles distant. Thus, in the Philippine Islands, at Camp Stotsenberg, in 386 cases of malaria studied by myself, only 98 were found infected with *Plasmodium vivax*, while at Camp Gregg, 40 miles distant from Camp Stotsenberg, Chamberlain, in 162 cases of malaria, found 83 infections with this plasmodium. At Camp Gregg the prevailing type of malaria was tertian, while at Camp Stotsenberg the prevailing type was due to *Plasmodium falciparum*, one of the species of æstivo-autumnal plasmodia.

In certain malarial regions in the tropics infections with *Plasmodium vivax* are very rare. Thus Leger and Baurý (1922) examined 96 natives in Guinea, East Africa, and did not find a single infection with this plasmodium, although 66 of these natives showed infection with other species of plasmodia. However, such a finding is very unusual, for it is a fact that even in the tropics infections with *Plasmodium vivax* form a very considerable proportion of all malarial infections, although in such regions infections with the æstivo-autumnal plasmodia generally far outnumber infections with either *Plasmodium vivax* or *Plasmodium malariae*.

**Incidence of Infection.**—Where malarial fevers are endemic a considerable proportion of the inhabitants, even though they present no clinical symptoms of the infection, will be found, upon examination, to be infected with *Plasmodium vivax* or other species of malaria plasmodia. In my experience, these latent infections are more frequently due to the æstivo-autumnal plasmodia than to *Plasmodium vivax*, but in many instances this plasmodium will be found in the blood of apparently healthy individuals.

As already stated, in temperate and in most subtropical regions where malaria is endemic the vast majority of both acute and latent infections are due to *Plasmodium vivax*, but in the tropics the æstivo-autumnal plasmodia furnish the greatest number of acute and latent infections. Thus, in Baltimore, Thayer and Hewetson observed 542 cases of malaria, of which 338 were due to *Plasmodium vivax*, and of 71 cases studied by Mannaberg, in Vienna, all but 10 were caused by this plasmodium. In Tonkin, Mathis and Leger (1909) found that of 344 malarial infections, 190, or 55 per cent., were due to *Plasmodium vivax*, while in Jerusalem Mühlens (1913) found that of 2,071 malarial cases, only 593, or 28.6 per cent., were caused by this plasmodium. In the tropical regions of Africa most observers have found that infections with *Plasmodium vivax* do not constitute more than 25 per cent. of the total malarial infections observed, and in many regions the percentage is even smaller. In the

Gulf States infections with *Plasmodium vivax* do not cause more than 40 to 45 per cent. of the total malarial infections observed, and in Central America and the tropical portions of South America infections with this plasmodium are less than 25 per cent. of the total malarial infections observed. In some tropical regions infections with *Plasmodium vivax* are very rare, as shown by Leger and Baurý (1922), who found no infections with this plasmodium in the natives of Guinea, East Africa, and only 9 infections with *Plasmodium vivax* in 155 cases of malarial infection observed at Dakar, East Africa. The incidence of infection with this plasmodium is greatest in the temperate regions where malaria is endemic, and least in the tropical malarial regions.

**Method of Transmission.**—*Plasmodium vivax* is transmitted from man to man by mosquitoes belonging to the *Anophelina*. That this plasmodium is the cause of tertian malaria was first proven by the direct inoculation into man of blood containing the parasite, and numerous observers were able to produce typical attacks of tertian malaria in this manner. In all such instances the plasmodia observed in the blood of the inoculated were identical with *Plasmodium vivax*, the species inoculated, in morphology and life-cycle, and the causative relation of the parasite to this type of malaria was thus proven beyond question.

In nature direct inoculation probably never occurs, and the plasmodium is transmitted entirely by suitable mosquitoes. Experimental proof of this fact has been furnished by numerous investigators who have allowed mosquitoes to bite individuals infected with *Plasmodium vivax*, and after a suitable period have allowed the same insects to bite healthy individuals, with the result that such individuals have developed tertian malaria, and the plasmodium has been found in their blood. Among those who have obtained positive results in such experiments with *Plasmodium vivax* may be mentioned Bignami, Bastianelli, Grassi, Manson, Rees, Buchanan, Schüffner, Jancso, Tsuzuki, Darling, King, and Mitzmain. All of these investigators have found that the blood of their experimentally infected cases contained plasmodia identical in morphology with *Plasmodium vivax*, and that in no instance was there any evidence that any mutation of species occurred.

The mosquitoes concerned in the transmission of *Plasmodium vivax* are all *Anophelina*, and experiments with other mosquitoes have invariably given negative results. A considerable number of species of the *Anophelina* have been experimentally proven to transmit *Plasmodium vivax*, and the following table, giving the species of mosquito, the name of the observer, the place and date of the observation, is of interest in this connection. It will be noted that no less than 32 species of anopheline mosquitoes have been proven to transmit this species of plasmodium.

*Mosquitoes Transmitting Plasmodium vivax, with Name of Observer, Place and Date*

Species of Mosquito	Name of Observer	Place	Date
<i>A. algeriensis</i> .	Sergents.	North Africa.	?
<i>A. albimanus</i> .	Darling.	Panama.	1909
<i>A. argyritarsis</i> .	G. de Faria.	Brazil.	1910
<i>A. barbirostris</i> .	Walker and Barber, Swellengrebel and Schüffner.	Malay States. Java.	1914 1919
<i>A. bifurcatus</i> .	Grassi.	Italy.	1898
<i>A. costalis</i> .	Many authors.	Ceylon and Tropical Africa.	
<i>A. crucians</i> .	King.	Louisiana.	1916
<i>A. culicifacies</i> .	Stephens and Christophers.	India.	1902
<i>A. funestus</i> .	Ross.	Gambia.	1900
<i>A. fuliginosus</i> .	Christophers.	India.	1916
<i>A. hispaniola</i> .	Sergents.	North Spain. North Africa.	?
<i>A. indefinitus</i> .	Barber.	Malay States.	1918
<i>A. intermedius</i> .	Chagas.	Brazil.	1908
<i>A. jesoensis</i> .	Christophers.	India.	1916
<i>A. kochi</i> .	Barber, Swellengrebel and Schüffner.	Malay States. Java.	1918
<i>A. leucosphyrus</i> .	Bais.	Java.	1919
<i>A. listoni</i> .	James.	India.	1902
<i>A. ludlowi</i> .	Banks.	Philippine Islands.	1907
<i>A. maculipennis</i> .	Grassi, Bastianelli and Bignami.	Italy.	1898
<i>A. mediopunctatus</i> .	Cruz.	Brazil.	1910
<i>A. minimus</i> .	Christophers.	India.	1916
<i>A. maculatus</i> .	Christophers.	India.	1916
<i>A. pharoensis</i> .	Newstead, Dutton.	Egypt.	1910
<i>A. plumbeus</i> .	Blacklock and Carter.	England.	1920
<i>A. punctipennis</i> .	King.	Louisiana.	1916
<i>A. quadrimaculatus</i> .	Thayer.	Maryland.	1900
<i>A. sinensis</i> .	Grassi and Celli.	Italy.	1898
<i>A. stephensi</i> .	Stephens and Christophers.	India.	1902
<i>A. superpictus</i> .	Grassi.	Italy.	1899
<i>A. tarsimaculata</i> .	Darling.	South America.	1909
<i>A. turkhudi</i> .	Stephens and Christophers.	India.	1902
<i>A. umbrosus</i> .	Barber.	Malay States.	1918

The life-cycle of the plasmodium within the mosquito has already been considered (Chapter XIII), and it will suffice to state here that the development of *Plasmodium vivax* within these insects does not differ, in general, from the development of the other malaria plasmodia in the mosquito.

**Experimental Infection of Lower Animals.**—Many attempts have been made to experimentally infect various species of lower animals with *Plasmodium vivax*, but in only one instance has success been claimed. Mesnil and Roubaud (1920) claim to have infected a young chimpanzee with this plasmodium, but their work has not been confirmed.

**Relation to Disease.**—As already stated, *Plasmodium vivax* has been proven beyond doubt to be the cause of that form of malarial fever known as tertian malaria, characterized by a paroxysm of chill, fever, and sweat-



ing occurring every forty-eight hours. The paroxysm is caused by the sporulation of the plasmodia, presumably by some toxin or toxins liberated at the time that the *merozoites*, or spores, become free in the blood serum. The destruction of the red blood corpuscles leads to the anæmia that is always present, while the liberation of the pigment produced within the plasmodia causes the pigmentation of the organs characteristic of malarial disease.

Sporulating plasmodia of this species may be easily found in the peripheral blood beginning from two to three hours before the period of 48 hours has elapsed until the termination of the chill, but even in this species of plasmodia the greater number of parasites sporulate in the vessels of the internal organs, especially the capillaries of the spleen.

The vast majority of infections with *Plasmodium vivax* are benign in character, but rarely pernicious symptoms may develop in infections with this parasite, and fatal results have been reported in a few instances. However, *Plasmodium vivax* usually gives rise to symptoms which, while most unpleasant, are not dangerous, and which readily yield to quinine. I have never observed a case of pernicious malaria due to this plasmodium, but well-authenticated cases are of record.

The incubation period of malaria due to *Plasmodium vivax* has been ascertained both after the inoculation of blood containing the plasmodium and after the bites of infected mosquitoes. As stated, the etiological relationship of *Plasmodium vivax* to tertian malaria was first demonstrated by the direct inoculation of the blood of individuals which contained the parasite into healthy individuals, and the incubation period varied, in the experience of different observers, from 6 to 21 days, the average period of incubation being from 11 to 12 days. The following table gives the results of several observers:

Observer	Plasmodium Inoculated	Period of Incubation	Type of Fever
Antolisei and Angelini.	<i>Plasmodium vivax.</i>	11 days.	Tertian.
Antolisei and Angelini.	<i>Plasmodium vivax.</i>	11 days.	Tertian.
Bein.	<i>Plasmodium vivax.</i>	12 days.	Tertian.
Bein.	<i>Plasmodium vivax.</i>	12 days.	Tertian.
Baccelli.	<i>Plasmodium vivax.</i>	6 days.	Tertian.
Mannaberg.	<i>Plasmodium vivax.</i>	21 days.	Tertian.

While the period of incubation observed after such experiments is of scientific interest and value, the data cannot be used as indicating the period of incubation after the inoculation of man by mosquitoes, for we have no evidence that infection with any of the malaria plasmodia ever occurs by the direct inoculation of blood containing the plasmodia. It is obvious that in the experimental production of malaria in this manner only the

forms of the plasmodium concerned in the human cycle of development undergo sporulation, as experiments have proven that the inoculation of susceptible individuals with blood containing only *gametes* never results in any evidences of infection, and the blood never shows any of the forms concerned in *schizogony* after such inoculations. In the inoculation of blood containing only the forms of the plasmodia belonging to the human life-cycle of the parasite it is reasonable to believe that the period of incubation will be shorter than that observed when the mosquito transmits the *sporozoites* to man. That this is true has been proven experimentally by many observers who have allowed mosquitoes infected with *Plasmodium vivax* to bite healthy individuals. The following table gives the results of some of these experiments :

Observer	Type or Mosquito Infection	Period of Incubation	Type of Fever
Bastianelli and Bignami.	<i>Plasmodium vivax</i> .	18 days.	Tertian.
Fearnside.	<i>Plasmodium vivax</i> .	14 days.	Tertian.
Fearnside.	<i>Plasmodium vivax</i> .	20 days.	Tertian.
Fearnside.	<i>Plasmodium vivax</i> .	24 days.	Tertian.
Schüffner.	<i>Plasmodium vivax</i> .	16 days.	Tertian.
Schüffner.	<i>Plasmodium vivax</i> .	16 days.	Tertian.
Jancso.	<i>Plasmodium vivax</i> .	15 days.	Tertian.

The table shows that the shortest period of incubation after the bite of mosquitoes infected with *Plasmodium vivax* was 14 days, and the longest period noted was 24 days, while the average period of incubation was 17.5 days. It will thus be seen that the period of incubation after the bite of infected mosquitoes experimentally is longer than after the direct inoculation of blood containing the plasmodium. Under natural conditions the period of incubation agrees practically with the period noted after experimental infection by the mosquito, for in military expeditions into localities in which infection with *Plasmodium vivax* is endemic it has been noted repeatedly that the first cases of infection with this parasite among the troops begin in about two weeks after reaching the infected district, and that the greatest number of infections are observed during the third week of residence in the district, provided no measures are taken to prevent infection. The period of incubation under natural conditions varies exceedingly, of course, depending very largely upon the resistance of the individual and the conditions present, as to the number of infected mosquitoes, exposure to unusual hardships, and many other factors, but in the vast majority of instances it will be found that the period of incubation agrees fairly well with the results obtained by the experimental production of the disease in man by the bite of infected mosquitoes.

While *Plasmodium vivax* is the cause of tertian malaria it should be remembered that many individuals are resistant to the toxin produced by

this parasite and never exhibit definite symptoms of the infection. These latent infections will be considered in the discussion of the prophylaxis of malaria. (See Chapter XVI.)

Subspecies I. *PLASMODIUM VIVAX MINUTUM*,  
Emin, 1914, *emend.*

Synonyms: *Plasmodium vivax*, var. ? Craig, 1900. *Plasmodium vivax*, var. *minuta*, Emin, 1914. *Plasmodium camaranense*, Ziemann, 1915. *Plasmodium ovale*, Stephens, 1922. *Plasmodium minutum* (Emin), Lane, 1923.

**History and Nomenclature.**—In the Report of the Surgeon General of the United States Army, for 1900, I described a malaria plasmodium occurring in the blood of United States soldiers returning from service in the Philippine Islands and suffering from a fever presenting tertian paroxysms indistinguishable clinically from the paroxysms caused by *Plasmodium vivax*. The marked difference in morphology between this plasmodium and *Plasmodium vivax* led me to regard it as either a distinct variety of *Plasmodium vivax* or a tertian plasmodium that had acquired the differential characteristics described through some unknown environmental condition. I did not give this plasmodium a name, as I was undecided as to its exact zoological position, and because I believed that further observations would decide whether or not it was entitled to be regarded as a new variety or species of plasmodium.

In 1914, Ahmed Emin described a plasmodium occurring in the blood of pilgrims at Camaran, in the Red Sea, near Hodeidah, which is undoubtedly identical with the plasmodium I described in 1900, and which he named *Plasmodium vivax*, var. *minuta*, and apparently the same plasmodium has been rediscovered by Stephens (1922) in the blood of a patient suffering from malaria acquired in East Africa. Although Stephens mentions the resemblance in morphology of his plasmodium to *Plasmodium vivax minutum*, he named it *Plasmodium ovale*. As a matter of fact, any unprejudiced reader who will compare the descriptions given by Emin, Stephens, and myself of the plasmodium observed by each must admit that these descriptions undoubtedly all refer to the same organism, and the colored illustrations published by Stephens leave no doubt in my mind that we observed and described the same parasite, and that this parasite is identical with Emin's plasmodium.

As noted by Lane (1923) the name suggested by Stephens, *Plasmodium ovale*, cannot stand, for if it is finally established that this plasmodium is entitled to specific rank the specific name given by Emin would be the correct one, and the plasmodium should be named *Plasmodium minutum* and not *Plasmodium ovale*.

Ziemann (1915) renamed Emin's plasmodium *Plasmodium camaranense*, but this name has no standing, as it was used simply because Ziemann

did not consider the name given by Emin to be a descriptive one, and such a reason is not a valid one for changing the name of any organism.

At the present time it is my belief that the plasmodium which is known as *Plasmodium vivax*, var. *minuta*, is a subspecies of *Plasmodium vivax*, and can be easily differentiated from the latter plasmodium. It is really purely a matter of opinion whether the plasmodium is considered as a subspecies or a species, and for those who believe it to be a species, the proper name is *Plasmodium minutum*. The important fact is, that this plasmodium is undoubtedly distinct from *Plasmodium vivax* in its morphology, and should be accepted as at least a subspecies of *Plasmodium vivax*, which it most closely resembles.

**Morphology.**—The following is my original description of this plasmodium as published in the Report of the Surgeon General of the United States Army, for 1900:

“ In studying the blood of soldiers returning from the Philippines and suffering from malaria, I have noticed in several cases a peculiar form of the malaria parasite which I have classified in reporting on cases as a tertian parasite, but which presents so many differences from the ordinary tertian parasite as to indicate that it is a distinct variety of the plasmodium.

“ I have invariably observed it in the blood of patients having tertian paroxysms, and always in large numbers. All stages of the parasite have been observed, from the hyaline disks to the segmenting bodies. It appears first as a very refractive circular hyaline disk within the erythrocyte, having a sharp outline and no amœboid motion. The absence of amœboid motion differentiates it from all other young forms of the malaria plasmodia. The hyaline disk is about one-fourth the size of the infected corpuscle, which is normal in size and appearance.

“ In a few hours the hyaline disk has grown to about one-half the size of the corpuscle which contains it and has become pigmented. The pigment exactly resembles that found in the ordinary tertian parasite, being finely granular and motile and distributed unequally throughout the parasite. The parasite is circular in shape and devoid of amœboid motion. The border of the parasite is very sharply defined, and the protoplasm is very refractive and sometimes finely granular. The infected corpuscle is unaltered in size and color, and is not crenated or shrunk.

“ In about thirty-eight to forty hours the parasite has nearly filled the corpuscle containing it and the amount of pigment has increased. The parasite is circular in shape and presents the same refractive protoplasm and sharply cut border; the pigment is sluggishly motile, reddish in color, and in the shape of fine granules. The infected corpuscle is normal in size and color.

“ The segmenting forms are much smaller than the ordinary tertian segmenting forms, and the greatest number of segments observed has



been ten. The segments are oval in shape or perfectly round, sharply cut and refractive. The pigment is usually collected in a solid reddish-brown mass at the centre of the segmenting parasite, and is non-motile."

In the discussion of the differential points between this plasmodium and the other malarial plasmodia, I said: "The differentiation of this parasite from the quartan organism is really much more difficult than is the case with the tertian or æstivo-autumnal plasmodia. Indeed, I mistook them for quartan parasites until the clinical histories of the cases and certain peculiarities of the parasites caused me to study them more carefully."

While in my original description I stated that amoeboid motility was absent in this plasmodium further observations have shown that it may be present, but it is always much less pronounced than in *Plasmodium vivax*, and one never observes the bizarre forms within the erythrocytes that are so characteristic of the latter species.

My original description was based upon the morphology of the plasmodium as seen in living, unstained preparations, but the study of specimens stained with the Wright stain confirmed Emin's description of the stained plasmodium observed by him.

Emin described the plasmodium as resembling the benign tertian plasmodium, *Plasmodium vivax*, but differing from it principally in its smaller size, lack of enlargement of the infected erythrocyte, and smaller number of spores or *merozoites*. He stated that the pigment resembles that of *Plasmodium vivax*, and that at the time of sporulation it almost filled the infected corpuscle, which was not enlarged, and that from four to ten *merozoites* were produced by the division of the parasite. He noted that amoeboid motility was slight as compared with *Plasmodium vivax*, and that multiple infection of the erythrocyte was frequently observed.

In preparations stained with Wright's stain the young "ring-forms" are indistinguishable from the ring-forms of other malaria plasmodia. There is the same ruby-red or violet dot or dots of chromatin, surrounded by an unstained area and enclosed by a ring of blue-stained cytoplasm, in the case of the *gametes*; the dot of chromatin being situated upon the periphery of the blue-stained cytoplasm, in the case of the *schizonts*. The infected red corpuscle stains normally and is sometimes oval in shape.

In half-grown forms the shape of the plasmodium is invariably oval or almost round, in stained preparations, and the chromatin is usually present as one or more irregularly shaped masses lying in the blue-stained cytoplasm. Generally the chromatin, even in these forms, tends to collect toward the periphery, while the pigment tends to collect toward the centre of the organism.

Many of the erythrocytes which contain parasites are oval in shape, a point which Stephens lays special stress upon, but others are perfectly circular, and this is true of every stage in the development of the plasmo-

dium, parasites being present in both oval and circular blood corpuscles. When the plasmodium is in an oval erythrocyte the shape of the parasite is also oval, but many plasmodia are observed, in every stage of development, which are circular in shape, and even in Stephens' colored drawings, many of the plasmodia are circular in shape and lie within circular erythrocytes.

In his original description Emin stated that he could not be sure whether Schüffner's dots occurred in the infected erythrocyte, as in infections with *Plasmodium vivax*, but, as stated in my later description (1914), Schüffner's dots do occur, although less regularly than in infections with *Plasmodium vivax*. In stained preparations the cells containing half-grown forms often show Schüffner's dots, and this fact serves to differentiate the plasmodium from the quartan plasmodium, *Plasmodium malariae*. As stated in my original description, I believe that those authors who have reported Schüffner's dots as occurring in the cytoplasm of erythrocytes infected with *Plasmodium malariae* have really been observing *Plasmodium vivax minutum*, for it is with the quartan plasmodium that this plasmodium is most apt to be confused. In the description of his plasmodium, *Plasmodium ovale*, Stephens states that Schüffner's dots are frequently observed.

In stained preparations the sporulating forms almost fill the infected erythrocyte, and each spore or *merozoite* consists of a round or oval mass of blue-stained cytoplasm and a ruby-red or violet dot of chromatin situated within or on the periphery of the cytoplasm. The number of spores or *merozoites* is given by Emin as from 4 to 10, by Stephens as from 6 to 12, while in my own experience, the number has varied from 6 to 10, the average being from 8 to 10.

That Stephens was observing the same plasmodium as Emin and myself is very evident from the language of his summary of the characteristics of the plasmodium observed by him. He says:

"The characteristics, then, of this parasite so far as concerns the medium forms are a non-amoeboid, pigmented, compact, round or oval parasite, resembling quartan, in a red cell showing Schüffner's dots, which is either normal in size or only slightly enlarged."

Stephens did not observe multiple infection of the erythrocytes to be common, as noted by Emin, nor any forms that resembled *gametes*. As he only studied this plasmodium in a single case of infection his failure to observe *gametes* is easily explained.

As regards the morphology of *Plasmodium vivax minutum*, both in unstained and stained preparations, it may be stated that in general it resembles *Plasmodium malariae*, the quartan plasmodium, except that it is often seen in erythrocytes which show Schüffner's granules or dots, and that the so-called "band forms," so characteristic of the quartan plasmodium, have not been observed. The number of *merozoites* is similar

to the number observed in *Plasmodium malariae*, but their arrangement is not so regular. The fact that this plasmodium is found only in cases showing a tertian periodicity should serve to distinguish it from *Plasmodium malariae*, which is associated with a fever occurring every seventy-two hours.

The gametes of *Plasmodium vivax minutum* are similar in their morphology in both unstained and stained preparations to those of *Plasmodium vivax*, but are much smaller, and the erythrocyte containing



FIG. 71.—*Plasmodium vivax minutum*. Stained with Wright's stain.  $\times 1,500$ . 1. Young "ring-form" or trophozoite. 2 and 3. Young schizonts. 4, 5 and 6. Half-grown schizonts. 7, 8, 9 and 10. Presporulating schizonts. 11 and 12. Sporulating schizonts. The dark areas are the chromatin of the nucleus. Note lack of enlargement of the infected red blood corpuscle and the presence of Schuffner's granules in the cytoplasm of the infected red corpuscles in 3, 4, 6, 8, 10 and 11. Note small number of spores or merozoites.

them is not enlarged, even when they are fully developed. The same difference in staining reaction is observed between the *microgametocytes* and *macrogametocytes* as noted in the male and female gametes of *Plasmodium vivax*.

**Habitat.**—This plasmodium lives in the red blood corpuscles of man and there undergoes *schizogony*. Its sexual life-cycle, or *sporogony*, is undoubtedly passed in some species of anopheline mosquito, but nothing has been done up to the present time to determine the species of mosquito or mosquitoes that are effective transmitters of this species of plasmodium.

**Occurrence in Lower Animals.**—There are no observations of record that indicate that this species of plasmodium occurs in any of the lower animals in nature, or that it is infective to any of the lower animals.

**Cultivation.**—*Plasmodium vivax minutum* has not been cultivated. It is probable that it could be cultivated in dextrose blood, but so far as I know, no efforts have been made to cultivate this species of plasmodium.

**Life-history.**—Only the human life-cycle of this plasmodium is known.

It undergoes its development in the erythrocytes of man and sporulates every 48 hours, producing from 4 to 12 *merozoites*, according to those observers who have had the opportunity of studying the parasite. The development of this species within the mosquito has not been observed, but it is undoubtedly similar to the development of other species of malaria plasmodia.

**Geographical Distribution.**—The geographical distribution of *Plasmodium vivax minutum* has not been fully ascertained, but cases of infection with it have been observed at Camaran, in the Red Sea, by Emin; a single case infected in East Africa, by Stephens; and several cases in soldiers of the U. S. Army returning from the Philippine Islands, by myself. I have also observed the same plasmodium in smears of blood obtained from patients suffering from malaria in Cuba, and a single case of infection in the Canal Zone. It is probable that this plasmodium is widely distributed, and that it is frequently mistaken for *Plasmodium malariae* by the ordinary observer.

**Incidence of Infection.**—It is evident, from the small number of cases of infection with *Plasmodium vivax minutum*, that it is a comparatively rare species, even in the regions from which it has been reported. In the examination of many hundreds of malarial cases in the Philippine Islands I encountered this plasmodium in only some half a dozen instances, although no special effort was made to ascertain the exact incidence of infection. I am inclined to believe that, had a careful search been made for the plasmodium in the localities in which the infections observed originated, other cases of infection with it would have been discovered, but it is certainly true that this plasmodium occurs much less frequently than any of the other species of human malaria plasmodia.

**Method of Transmission.**—There is nothing definite known regarding the method of transmission of this plasmodium, as no work has been accomplished regarding the mosquito transmission of the parasite. However, there is no reason to doubt that, like the other species of malaria plasmodia, this species is transmitted from man to man by one or more species of mosquitoes belonging to the *Anophelinae*.

**Experimental Infection of Lower Animals.**—There have been no observations published regarding the possibility of transmitting *Plasmodium vivax minutum* to any of the lower animals, but, reasoning from analogy, there is little doubt that this species is confined to man, and does not occur in the lower animals.

**Relation to Disease.**—There is no experimental evidence that *Plasmodium vivax minutum* is a pathogenic parasite, as infection with this species has never been produced in man, either by the direct inoculation of blood containing the parasite into a healthy individual or through the bites of infected mosquitoes. There is no reason to doubt that such experiments



would prove successful, but up to the present time no one has published the results of such experiments, if they have been made. The evidence of the pathogenic nature of this plasmodium rests upon the fact that it has been found in the blood of patients suffering from attacks of fever occurring every 48 hours and accompanied by the other clinical symptoms that are characteristic of a malarial paroxysm, and that the parasite has not been found in the blood in other diseases or in healthy individuals. No doubt latent cases of infection with this species exist, as in the case of the other malaria plasmodia, but up to the present time all the instances of infection with this parasite that have been reported have shown clinical symptoms of the infection, and there is no reason to doubt that *Plasmodium vivax minutum* was the cause of the clinical condition noted.

### Species II. PLASMODIUM MALARIÆ, Laveran, 1881.

Synonyms: *Oscillaria malarie*, Laveran, 1881, *pro parte*. *Plasmodium*, var. *quartana*, Golgi, 1890. *Hæmameba malarie*, Grassi et Feletti, 1890. *Plasmodium malarie*, var. *quartana*, Celli et Sanfelice, 1891. *Hæmameba laverani*, var. *quartana*, Labbé, 1894. *Hæmosporidium quartana*, Lewkowitz, 1897. *Plasmodium malarie quartanum*, Labbé, 1899. *Hæmomonas malarie*, Ross, 1900. *Plasmodium golgii*, Sambon, 1902. *Plasmodium quartana*, Billet, 1904. *Hæmameba malarie*, var. *quartana*, Laveran, 1901. *Plasmodium quartana*, Celli, 1904.

**History and Nomenclature.**—Although Laveran undoubtedly saw and partially described *Plasmodium malarie* in 1881, he did not recognize it as a distinct species, and to the Italian students of malaria, Marchiafava and Celli, Golgi, and Antolisei, we owe the differentiation of this species from the benign tertian plasmodium, *Plasmodium vivax*, and the æstivo-autumnal plasmodium, *Plasmodium falciparum*. Golgi (1886) gave the first complete description of this plasmodium, and followed every stage of the *schizogony* in the blood of the 22 cases of infection with the plasmodium which he studied. He demonstrated that the chill and fever occurred at the time of the sporulation of the plasmodium, which occurred every 72 hours, and called attention to the fact that triple infections with the plasmodium gave rise to daily paroxysms and a quotidian fever. Golgi's observations were confirmed by Osler in the same year, and have since been confirmed by every observer who has studied this species of plasmodium.

*Plasmodium malarie* is a rare species as compared with either *Plasmodium vivax* or *Plasmodium falciparum* and is the cause of quartan malarial fever. It can be easily differentiated from other species of malaria plasmodia, both by the clinical symptoms that it produces in man and by its morphology.

**Morphology of Plasmodium Malarie in Unstained Preparations.**—*Plasmodium malarie* completes its cycle of development in man in 72 hours, giving rise to a type of malarial fever characterized by a chill and

fever occurring upon every fourth day. Like *Plasmodium vivax*, it appears at first within the infected erythrocyte as a small amœboid body, the *trophozoite*, which has a ring-like shape and cannot be distinguished from other species of malaria plasmodia at this early stage. Within a few hours, however, and before the development of pigment, the *trophozoite* of *Plasmodium malariae* becomes more refractive in appearance, more sharply cut in outline, and less actively amœboid than the corresponding stage of *Plasmodium vivax*, and can be distinguished from the latter organism by attention to these details of morphology in the unstained preparations. The infected red corpuscle is not enlarged, and is slightly more greenish in color than the normal red cells. The *trophozoites* measure from 1.5 to 2 microns in diameter at this stage in development.

Within six hours after infecting the red blood corpuscle the *trophozoite* of *Plasmodium malariae* begins to show pigment within the cytoplasm and becomes a *schizont*. The pigment is in the form of a few rather coarse granules of a dark-brown color, situated near the periphery of the angular or roughly rounded parasite, and is very slightly motile, thus differing from the pigment in *Plasmodium vivax*, which is in the form of very fine granules, reddish brown in color, and very actively motile. At this early pigmented stage the *schizont* is very sluggish in its amœboid movements and the infected red blood corpuscle is not enlarged, but even slightly smaller than normal.

The *schizont* slowly increases in size and loses its amœboid motility; the pigment increases in amount and collects at the periphery of the organism in the form of large, very dark-brown grains which are motionless. The cytoplasm of the organism is very distinct, hyaline in appearance, and often appears slightly granular. The infected red blood corpuscle is normal in size or slightly smaller than normal, and appears a darker green in color.

At the end of 24 hours the distinguishing features of the quartan plasmodium are fully developed. The parasite is sharply outlined, is very refractive, and its cytoplasm presents a peculiar finely granular appearance; the pigment is dark brown in color, immotile, and arranged around the periphery, sometimes forming an almost perfect wreath, or, more rarely, is collected in small, irregular masses within the cytoplasm. The shape of the plasmodium at this stage of development is usually triangular, oval, or spherical, and the bizarre forms, so characteristic of *Plasmodium vivax*, and due to the active amœboid motility of the latter parasite, are never observed. The plasmodium occupies less than half of the infected erythrocyte, which is generally a little smaller than normal and darker green in color. Amœboid motion is very slight.

At the end of about 36 hours all of the phenomena noted above are more pronounced, and the parasite fills about two-thirds of the infected

red blood corpuscle. The pigment has increased in amount and is now collected in irregular masses near the periphery. Amœboid motion is entirely absent. The growth of *Plasmodium malariae* from this time on is very slow, and pre-sporulating forms may be observed in the peripheral blood as early as 24 hours before the time of sporulation is due, *i.e.*, before 72 hours have elapsed, thus showing that the process of sporulation, or *schizogony*, is slower than in *Plasmodium vivax*. At this stage in the development of the *schizont*, the so-called "band-forms" are frequently observed, and are characteristic of this species of plasmodium. They appear as a broad band of hyaline cytoplasm, which contains pigment, and which stretches directly across the infected erythrocyte. Such forms are very prominent in stained preparations, but may be easily overlooked in unstained specimens, being mistaken for degenerated red blood corpuscles. As growth continues the pigment increases in amount and becomes scattered through the cytoplasm in an irregular manner. The infected erythrocyte appears shrunken, and is almost always smaller than those surrounding it, and darker green in color.

In many instances sporulation begins before the infected erythrocyte is filled by the plasmodium, and sporulating forms may be seen within erythrocytes which are not entirely filled by them. Sporulation occurs both in the vessels of the internal organs and in the peripheral blood, and such forms may be observed in the peripheral blood at the end of from 70 to 72 hours and sometimes earlier. The vast majority of the plasmodia sporulate at the end of 72 hours. At this time the plasmodium fills the infected red blood corpuscle, and sporulation occurs in the following manner:

The pigment becomes collected at the exact centre of the parasite in a solid, almost black, spherical mass, or in a star-like arrangement distributed from the centre, and about the same time all trace of the infected erythrocyte disappears, the plasmodium apparently being extracellular; in a short time radial striations are observed shooting out from the pigmented centre, and these, joining at their extremities, form from 6 to 14 oval segments or spores, the *merozoites*. As a rule, the number of spores varies from 8 to 10, and it is seldom that more than 12 are observed, and the latter number occurs very infrequently. The *merozoites* are generally arranged in a symmetrical manner around the central pigment in a single row, giving the so-called daisy or "Marguerite" appearance to the plasmodium that is quite characteristic of the sporulating forms of *Plasmodium malariae*. The number and the beautifully regular arrangement of the *merozoites* would alone serve to distinguish this stage of the quartan from the corresponding stage of the tertian plasmodium, but the quartan *merozoites* are also more oval in shape, more refractile, and slightly larger, measuring about 2.5 microns in diameter.

When sporulation is complete each *merozoite* becomes free in the



blood plasma and, in the human cycle, again invade the red blood corpuscles, some becoming *schizonts*, while others become *gametes*.

At the time of sporulation the infected red blood corpuscle consists of a narrow rim of cytoplasm surrounding the plasmodium, and there is no enlargement of the cell; indeed, the infected erythrocyte is generally smaller than normal. When sporulation is completed the infected corpuscle disappears and the pigment is liberated and may appear as free pigment in the preparation. There is a slight difference in the size of the sporulating plasmodia, and rarely sporulating forms are observed so small as to fill only two-thirds of the infected erythrocyte, but as a general rule the sporulating forms of *Plasmodium malariae* are remarkably uniform in size, unlike those of *Plasmodium vivax*, which vary considerably in this respect.

The *gametes* of *Plasmodium malariae*, in unstained preparations, resemble very closely the *schizonts*, but the *gamete* never is seen as a "band-form," as is the *schizont*. *Gametes* are never numerous in the peripheral blood and often are so scarce, even in old infections, as to require a long search in order to discover one or two. They are slightly larger than the *schizonts*, and the female form is considerably larger. The *macrogametocyte* contains much coarse dark-brown pigment, while the *microgametocytes* are less heavily pigmented. Both are usually distinctly oval in shape, which distinguishes them from the quartan *schizonts*, and both, when fully developed, entirely fill the infected red blood corpuscle. The *gametes*, both male and female, are easily distinguished from those of *Plasmodium vivax* by their smaller size and the fact that they occur within erythrocytes that are not appreciably enlarged. The *gametes* of *Plasmodium malariae* can be best recognized in stained preparations of blood, as their close resemblance to the *schizonts* in unstained preparations renders their recognition very difficult unless one has had a large experience in the study of this plasmodium.

**Morphology of Plasmodium Malariae in Stained Preparations.**—The staining reactions of *Plasmodium malariae* with the Wright stain, or other modification of the Romanowsky method, are the same as those of *Plasmodium vivax*, already described, the cytoplasm staining blue, the chromatin of the nucleus a red or violet, while the vesicular portion of the nucleus remains unstained.

The youngest stage of development, the ring-form, or *trophozoite*, consists of a blue-stained ring of cytoplasm which contains a milky-appearing spherical or oval area in which lies, at some portion of the periphery of the ring, a bright red or violet dot of chromatin. The unstained area, or vacuole, is observed only in those *trophozoites* that are to become *schizonts*, and disappears in the *schizont* at a very early stage in its development. In fact, it is only in the ring-forms that the unstained portion of



the nucleus is plainly visible. The cytoplasm of *Plasmodium malariae*, even in the youngest *trophozoites*, stains a more intense blue color than does the cytoplasm of *Plasmodium vivax*, and in the ring-forms, or *trophozoites*, the chromatin, which stains a deep red, is situated nearer the centre of the unstained vesicular portion of the nucleus.

The young *schizont* consists of a triangular, oval, or round mass of blue-stained cytoplasm containing one or more irregular collections of deep-red chromatin, and a considerable amount of pigment which appears greenish black in well-stained preparations. If the blood preparations be taken and stained at the end of 48 hours numerous examples of the characteristic "band-" or "ribbon-form" of the quartan *schizont* will be present. Such forms consist of a band of dark-blue-stained cytoplasm stretching across the infected erythrocyte, and enclosing a mass of chromatin stained a deep-red color. I have never observed these forms of the *schizont* in any other variety of malarial infection, and a diagnosis of infection with *Plasmodium malariae* is justified when such forms are present in a preparation.

The staining reactions of the *schizont* during later stages of development are similar to those of *Plasmodium vivax*, except that the unstained area is lost early in the development of the *schizont* and that the cytoplasm and chromatin stain more intensely. The latter, when distributed throughout the cytoplasm, as it is in the older *schizonts*, is in the form of larger and thicker filaments, collected in more definite clumps, and staining a deeper red, than the chromatin in corresponding stages of development of *Plasmodium vivax*, and in the pre-sporulating and sporulating *schizonts* the chromatin is collected in more compact masses than in *Plasmodium vivax*. Because of their deeper staining and the oval or round form of the *schizonts* of *Plasmodium malariae*, their outline is much more distinct in stained preparations than in the case of the tertian plasmodium, and in the nearly full-grown *schizont*, the infected erythrocyte is plainly visible as a pink-stained border surrounding the plasmodium.

In the sporulating forms of *Plasmodium malariae* the pigment stains a greenish black, and is collected in a compact mass at the centre of the parasite or in a star-like manner radiating from a mass at the centre of the organism. The spores, or *merozoites*, usually numbering from 8 to 10, are arranged in a circular manner around this mass of pigment, and consist of a ring of blue-stained cytoplasm encircling an unstained area which contains a small circular deep-red mass of chromatin. The sporulating *schizonts* of this species are very characteristic, and, once seen, can hardly be mistaken for those of any other species of plasmodium, with the possible exception of the sporulating forms of *Plasmodium vivax minutum*.

When liberated by the breaking up of the infected erythrocyte each *merozoite*, in stained preparations, consists of a deep-blue mass of cyto-

plasm and of a compact mass or dot of dark-red chromatin situated to one side of the centre of the organism. The chromatin is usually surrounded



FIG. 72.—*Plasmodium malariae*. The quartan malaria plasmodium. (Photomicrographs. Army Medical School Collection.) Stained with Wright's stain. 1. Young parasites, the so-called "ring-forms."  $\times 1,500$ . 2. Half-grown schizont, a typical so-called "band-form."  $\times 1,800$ . 3. Three-quarters grown schizont.  $\times 1,500$ . 4. Presporulating schizont.  $\times 1,800$ . Note lack of enlargement of infected red blood corpuscle.

by a very small unstained area, the vesicular portion of the nucleus. The merozoites of *Plasmodium malariae* are slightly larger than those of *Plas-*

*modium vivax*, measuring from 1.75 to 2.25 in diameter, and stain much more intensely.

In stained preparations the *gametes* of *Plasmodium malariae* resemble very closely those of *Plasmodium vivax* except that they are smaller, more richly pigmented, and are not found in greatly enlarged erythrocytes. They also resemble the *schizonts* of *Plasmodium malariae*, but may be distinguished readily by one trained in the study of the parasite. The young *macrogametocyte* contains more pigment and less chromatin than a *schizont* of the same size, while the young *microgametocyte* contains less pigment and more chromatin. The staining reactions are the same as in the gametes of *Plasmodium vivax*, the male, or *microgametocyte*, staining a pale-blue or greenish-blue color, while the female, or *macrogametocyte*, stains a deep-blue color. The staining reactions and arrangement of the chromatin in the *macro-* and *microgametocytes* of *Plasmodium malariae* are similar to those of *Plasmodium vivax*, and one description answers equally well for both parasites. (See page 400.)

The *microgametes* of *Plasmodium malariae* appear in stained preparations as long threadlike filaments of pink or red stained chromatin similar in morphology to the *microgametes* of *Plasmodium vivax*. It is very rarely that one is fortunate enough to secure stained preparations showing *microgametes*, owing to the fact that *microgametocytes* are very scarce in the peripheral blood of patients suffering from infection with this plasmodium.

In stained preparations of blood containing *Plasmodium malariae*, the infected red blood corpuscle stains a light-pink or orange-pink color, and is either normal in size or slightly smaller than normal. The pink granulations known as Schüffner's dots or granules, so characteristic of the red corpuscles infected with *Plasmodium vivax*, are never observed in the corpuscles infected with *Plasmodium malariae*, and the cells infected with this species of malaria plasmodium do not show any form of granular degeneration. Some authors have reported the occurrence of Schüffner's granules in the infected corpuscles in isolated cases of infection with *Plasmodium malariae*, but, since the differentiation of *Plasmodium vivax* *minutum* as a distinct species, I believe that all such instances can be explained by the observer having mistaken this species for *Plasmodium malariae*, a mistake that is very easily made because of the very close resemblance of *Plasmodium vivax* *minutum* in size and general morphology to *Plasmodium malariae*. As Schüffner's granules occur in many of the erythrocytes infected with *Plasmodium vivax* *minutum*, and as the infected cell is not enlarged, one can readily understand why such an error is possible.

The lack of enlargement of the infected red blood corpuscle and the

absence of Schüffner's granules serve to distinguish *Plasmodium malariae* from *Plasmodium vivax* and from *Plasmodium vivax minutum* in stained

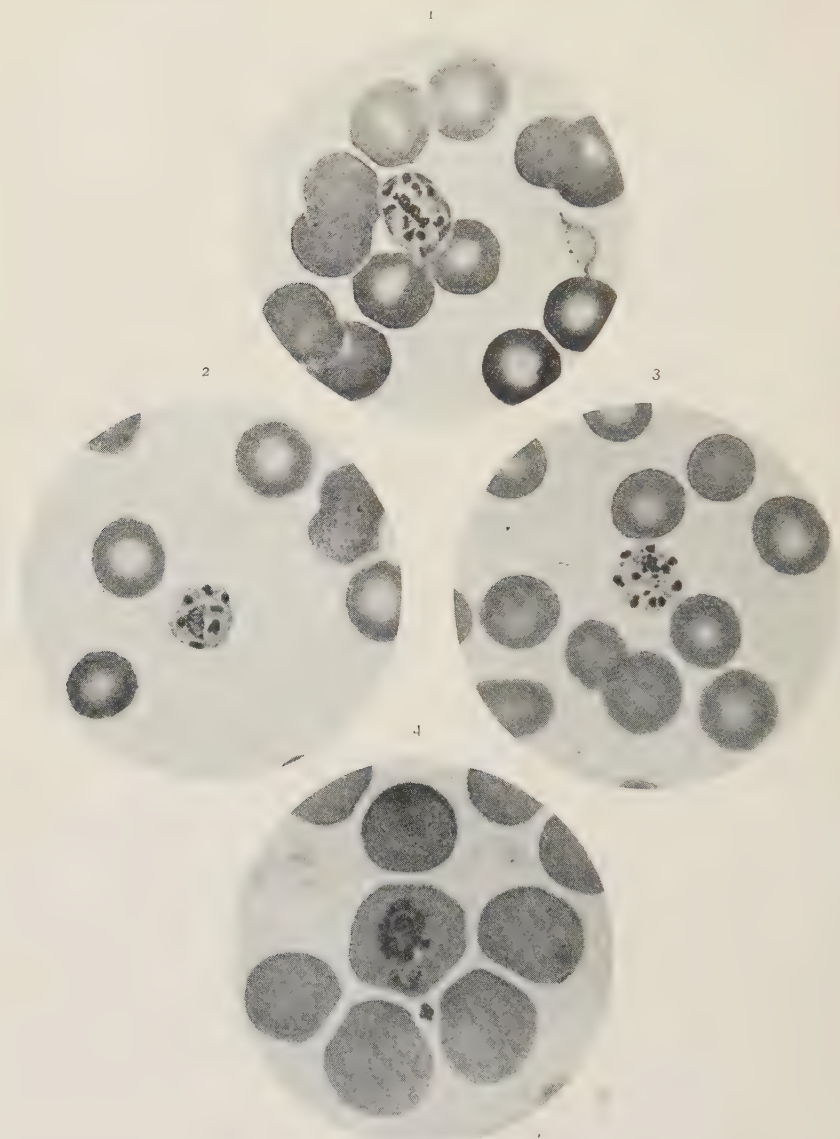


FIG. 73.—*Plasmodium malariae*. Quartan plasmodium. (Photomicrographs. Army Medical School Collection.) Wright's stain. 1. Presporulating plasmodium.  $\times 1,500$ . 2. Sporulating schizont.  $\times 1,200$ . 3. Sporulating schizont.  $\times 1,200$ . 4. Young gamete.  $\times 1,800$ . Note lack of enlargement of the infected red blood corpuscle and small number of spores, or merozoites.

preparations. In infections with *Plasmodium vivax* the infected erythrocyte is greatly enlarged and Schüffner's granules are present, while in



infections with *Plasmodium vivax minutum* the infected erythrocyte is not enlarged, but Schüffner's granules are often present.

**Habitat.**—*Plasmodium malariae* is a parasite of the erythrocytes of man, so far as is known, although one or two authorities have reported finding plasmodia of similar morphology in certain monkeys and apes. All efforts to inoculate this plasmodium into the lower animals have given negative results, and there is, at present, no reason for believing that this species is a parasite of any of the lower animals.

**Species Occurring in Lower Animals.**—Reichenow (1920) has reported the occurrence of a plasmodium apparently identical with *Plasmodium malariae* in two of six chimpanzees examined in the Cameroons. The forms resembling *Plasmodium malariae* were present in conjunction with forms resembling *Plasmodium vivax* and *Plasmodium falciparum*.

In 1922, Blacklock and Adler described forms resembling *Plasmodium malariae* in a chimpanzee, and here, also, it was found that forms resembling *Plasmodium falciparum* and *Plasmodium vivax* were present at the same time in the blood of the animal. In view of these findings it is probable that all the forms belonged to one species, which may be a parasite of the chimpanzee, rather than that all three species common to man were present in the animals. Much more research is necessary before it can be stated that *Plasmodium malariae* occurs in the lower animals, although there is no doubt that the work of Reichenow and Blacklock and Adler prove that forms do occur in the blood of the chimpanzee in nature that are practically identical in morphology with this species.

**Cultivation.**—*Plasmodium malariae* was first cultivated *in vitro* by Bass (1911). His observations have been confirmed by others, and this species appears to be as easily cultivated as the other species of malaria plasmodia.

**Life-history.**—The life-history of *Plasmodium malariae* is like that of the other malaria plasmodia, *schizogony* occurring in the blood corpuscles of man and *sporogony* in certain species of anopheline mosquitoes. The development in the blood corpuscles of man is completed in 72 hours, while the life-cycle in the mosquito, under favorable conditions of moisture and temperature, is completed in from 18 to 21 days. (See Chapter XIII.)

**Geographical Distribution.**—*Plasmodium malariae* is found more frequently in temperate and subtropical regions than in the tropics, but it is not so widely distributed a species as is *Plasmodium vivax*, and there are many malarial regions where this species has never been found. Even where it occurs it is generally a rare species, although there are some localities where it is the prevailing species. In the United States, *Plasmodium malariae* is most frequently encountered in the malarial parts of the Gulf States, while it is practically unknown in the Northern States, where *Plasmodium vivax* is the common cause of malarial fever. How-

ever, even in the regions where it occurs, hundreds of infections with *Plasmodium vivax* or *Plasmodium falciparum* will be observed to one of infection with *Plasmodium malariae*, so that it may be stated that the latter species is rare in the United States, and never causes any large proportion of malarial infections.

In Cuba, Panama, and Central America infections with *Plasmodium malariae* are observed, but they are rare as compared with infections with either *Plasmodium vivax* or *Plasmodium falciparum*, and this statement is true of the malarial regions of South America. In certain parts of Europe *Plasmodium malariae* occurs rarely, but in Italy and Greece this species is quite frequently encountered. It is found in Macedonia, Palestine, Ceylon, the Malay States, and in most of the malarial portions of Africa. In the Philippine Islands it is a comparatively rare species, and in the hundreds of cases of malarial infection that I studied there I observed this species in only eighteen patients. It may be stated that *Plasmodium malariae* has a world-wide distribution, but that it is a rare species, and there are many intensely malarial localities where it is never observed.

**Incidence of Infection.**—As already stated, *Plasmodium malariae* is one of the rare species of malaria plasmodia in most regions where malaria is endemic, and it is generally stated to be the most rare species of malaria plasmodia. This statement is incorrect, for the quotidian variety of the æstivo-autumnal plasmodium, *Plasmodium falciparum*, is much less frequently observed, and this is also true of the *minutum* variety of *Plasmodium vivax*. However, *Plasmodium malariae* is a rare species, as is well shown in the following table:

*Infections with Plasmodium malariae*

Observer	Locality	Cases Observed	Infections with <i>P. malariae</i>
Finot.	Blida.	4,211	26
Maillot.	Algeria.	2,338	21
Laveran.	Algiers.	311	7
Thayer and Hewetson.	Baltimore.	1,680	15
Griesinger.	Tübingen.	414	3
Mannaberg.	Vienna.	144	8
Craig.	Cuba and the Philippines.	Over 5,000	32

A consideration of the above table shows that infections with *Plasmodium malariae* are very rare in the experience of the observers mentioned, but other observers have reported much larger numbers of infections with this species, which is explained by the fact that they were situated in localities where this plasmodium was more commonly encountered. Thus, Leger and Baurý (1922), in Guinea, East Africa, examined 96 natives, and found *Plasmodium malariae* in the blood of 38, and *Plas-*

*modium falciparum* in the blood of only 27, thus proving that *Plasmodium malariae* was the most common species of plasmodium in that particular region. At Dakar, the same observers examined the blood of 366 natives, and found 155 infected with malaria plasmodia. Of these, no less than 42, or 27 per cent., were infected with *Plasmodium malariae*, while 104, or 47 per cent., were infected with *Plasmodium falciparum*, and only 9, or 5.8 per cent., were found infected with *Plasmodium vivax*. It is thus evident, that while in most regions, and in the experience of most investigators, *Plasmodium malariae* is a rare species, there are regions where it is common and, indeed, may be the prevailing species of plasmodium, and the most frequent cause of malarial infection.

**Method of Transmission.**—*Plasmodium malariae* is transmitted from man to man by mosquitoes belonging to the *Anophelinæ*. That this is true has been proven experimentally by numerous investigators, by allowing mosquitoes that have bitten individuals having this species of plasmodia in their blood to bite healthy individuals after a certain period of time has been allowed for the completion of the life-cycle of the parasite in the mosquito. Healthy individuals thus bitten have developed typical quartan malaria, and *Plasmodium malariae* has been found in their blood. Among those who have been successful in such experiments may be mentioned Bignami, Grassi, Buchanan, James, Stephens, and Christophers.

The mosquitoes transmitting *Plasmodium malariae* all belong to the *Anophelinæ*, and efforts to infect other mosquitoes have invariably given negative results. Owing to the rarity of this species of plasmodium, not so much work has been accomplished in determining the species of mosquitoes that may transmit *Plasmodium malariae* as in the case of *Plasmodium vivax* or *Plasmodium falciparum*, but a considerable number of species of *Anopheles* have been proven to transmit this plasmodium, as shown in the following table:

*Mosquitoes Transmitting Plasmodium malariae, with Name of Observer, Place, and Date*

Mosquito	Observer	Place	Date
<i>A. algierensis.</i>	Sergents.	Algeria.	1906
<i>A. costalis.</i>	Ross, Annett, Austin.	Africa.	1900
<i>A. culicifacies.</i>	Stephens and Christophers.	India.	1902
<i>A. funestus.</i>	Ross, Annett, Austin.	Gambia.	1900
<i>A. listoni.</i>	James.	India.	1902
<i>A. fuliginosus.</i>	Stephens and Christophers.	India.	1902-14
<i>A. maculipennis.</i>	Grassi, Bastianelli, Bignami.	Europe.	1898-99
<i>A. ludlowi.</i>	Banks.	India.	1907
<i>A. quadrimaculatus.</i>	Beyer, Pothier, Couret.	Louisiana.	1902
<i>A. rossi.</i>	Stephens and Christophers.	India.	1902
<i>A. sinensis.</i>	Grassi.	Italy.	1898
<i>A. stephensi.</i>	Stephens and Christophers.	India.	1902
<i>A. theobaldi.</i>	Stephens and Christophers.	India.	1902

From the above table it will be noted that thirteen species of anopheline mosquitoes have been proven to transmit *Plasmodium malariae*, but that only one species, *Anopheles quadrimaculatus*, transmits the quartan plasmodium in the United States. However, this is a very common species of mosquito, and the fact that it transmits the plasmodium does not explain the comparative rarity of this species. One would expect infections with *Plasmodium malariae* to be very common when the species is transmitted by so common a mosquito, but such is not the fact, and the exact reason for the rarity of infections with this plasmodium has not yet been ascertained. In India the plasmodium is transmitted by several species of mosquitoes, but in most localities infections with it are much less numerous than infections with either *Plasmodium vivax* or *Plasmodium falciparum*.

The life-cycle of *Plasmodium malariae* in the mosquito is similar, in its general features, to the life-cycle of other species of plasmodia, and has already been described. (See Chapter XIII.)

**Experimental Infection of Lower Animals.**—All attempts to infect the lower animals with *Plasmodium malariae*, either by the direct inoculation of blood containing the parasite or by the bites of infected mosquitoes, have resulted negatively.

**Relation to Disease.**—It has been proved beyond all doubt that *Plasmodium malariae* is the cause of that form of malarial disease known as quartan malarial fever, characterized by a paroxysm of chill, fever, and sweating occurring every 72 hours in uncomplicated infections. If three generations of the plasmodia are present a quotidian fever results, while if two generations are present the temperature may be irregular or paroxysms occur upon two successive days, followed by a fever-free interval of one day. This plasmodium produces the same destruction of the red blood corpuscles as does *Plasmodium vivax* and the other malaria plasmodia, so that severe anæmia is one of the prominent symptoms of the infection, while pigmentation of the organs occurs as with the other malaria plasmodia.

Most of the infections with *Plasmodium malariae* are benign in character, although fatal cases of infection with this species have been observed. Personally, I have never seen a fatal infection with this parasite, but some of the cases observed have been accompanied by severe symptoms, and have been very resistant to treatment. In most instances quinine, in proper dosage, will quickly control the active symptoms, but a long-continued course of the drug is generally necessary to cure the infection.

The incubation period of malaria due to *Plasmodium malariae* has been determined both after the direct inoculation of blood containing the parasite and after the bites of infected mosquitoes. The etiological relationship of this plasmodium to quartan malaria was first determined by the



direct inoculation of blood containing the plasmodia into healthy individuals, and it was found that the period of incubation of the disease when produced in this manner varied from 10 to 18 days, the average period of incubation being about 14 days. The following table gives the results obtained in such experiments by several observers:

*Period of Incubation After the Direct Inoculation of Blood Containing Plasmodium malariae*

Observer	Plasmodium Inoculated	Period of Incubation	Type of Fever
Gualdi and Antolisei.	<i>Plasmodium malariae</i> .	10 days.	Quartan.
Gualdi and Antolisei.	<i>Plasmodium malariae</i> .	12 days.	Quartan.
Gualdi and Antolisei.	<i>Plasmodium malariae</i> .	15 days.	Quartan.
Di Mattei.	<i>Plasmodium malariae</i> .	18 days.	Quartan.
Di Mattei.	<i>Plasmodium malariae</i> .	11 days.	Quartan.
Calandrucio.	<i>Plasmodium malariae</i> .	18 days.	Quartan.
Baccelli.	<i>Plasmodium malariae</i> .	12 days.	Quartan.

As in the case of *Plasmodium vivax*, the incubation period after the direct inoculation of blood containing *Plasmodium malariae* is considerably shorter than the incubation period after the bite of infected mosquitoes. It has been shown by several observers that the incubation period after the bite of infected mosquitoes averages nearly three weeks for *Plasmodium malariae*, while the average period of incubation after the direct inoculation of blood containing this species is about two weeks.

The period of incubation of malaria due to *Plasmodium malariae* in nature has not been determined, owing to the rarity of this species and consequent lack of opportunity for careful observations. In all probability the natural period of incubation is that observed experimentally after the bite of infected mosquitoes, but will depend, of course, upon the number of bites by infected insects and the dosage of *sporozoites*. As in infections with the other malaria plasmodia latent infections with this parasite are observed in which no symptoms of disease are present although the plasmodium may be demonstrated in the peripheral blood. The subject of latent infection is considered in the discussion of the prophylaxis of malaria. (See Chapter XVI.)

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## CHAPTER XV

### THE ÆSTIVO-AUTUMNAL MALARIA PLASMODIA. *PLASMODIUM FALCIPARUM*. *PLASMODIUM FALCIPARUM QUOTIDIANUM*. DOUBTFUL SPECIES OF MALARIA PLASMODIA. *PLASMODIUM TENUE*. *PLASMODIUM OVALE*.

The æstivo-autumnal malarial fevers are caused by certain species of plasmodia that differ markedly in their morphology and effect upon man from the plasmodia causing tertian and quartan malarial fever, and most fatal infections are due to the æstivo-autumnal plasmodia. There are two parasites concerned in the etiology of these fevers, *Plasmodium falciparum*, the common species, and the cause of subtertian or malignant tertian malaria, and *Plasmodium falciparum quotidianum*, a comparatively rare parasite, the cause of quotidian æstivo-autumnal malaria. *Plasmodium falciparum quotidianum* is usually considered a subspecies of *Plasmodium falciparum*, but some authorities consider it a distinct species. If it be decided that this plasmodium is entitled to full specific rank the name will have to be changed, but until the evidence is sufficient to place it upon an unquestioned specific basis I believe that it is preferable to regard it as a subspecies of *Plasmodium falciparum*.

#### Species III. *PLASMODIUM FALCIPARUM*, Welch, 1897.

Synonyms: *Oscillaria malariae*, Laveran, 1884, *pro parte*. *Plasmodium malariae*, var. *quotidianæ*, Celli and Sanfelice, 1891. *Hæmamaeba præcox*, Grassi and Feletti, 1890. *Laverania malariae*, Grassi and Feletti, 1890. *Hæmamaeba malariae præcox*, Grassi and Feletti, 1892. *Hæmamaeba laverani*, Labbé, 1894. *Hæmatozoon falciparum*, Welch, 1897. *Hæmonas præcox*, Ross, 1899. *Plasmodium malariae præcox*, Labbé, 1899. *Plasmodium præcox*, Blanchard, 1900. *Hæmamaeba malariae parva*, Laveran, 1900. *Plasmodium immaculatum*, Schaudinn, 1902. *Laverania præcox*, Nocard and Leclainche, 1903. *Plasmodium falciparum*, Blanchard, 1905. *Plasmodium falciparum subtertianum*, Bates, 1913. *Plasmodium perniciosum*, Ziemann, 1915. *Plasmodium caucasicum*, Marzinowsky, 1916.

**History and Nomenclature.**—The crescentic-shaped *gametes* of *Plasmodium falciparum* were first described by Laveran, in 1881, and it is probable that he also saw the young *trophozoites*, or “ring-forms,” but he did not regard these forms as belonging to a distinct species, but considered them as forms belonging to the life-cycle of a species which he named *Oscillaria malariae*, which we now know really consisted of at least three distinct species. It was not until 1885, when Golgi called attention to the probably distinct type of the crescentic and ovoid parasites, and suggested the possibility of their belonging to a new species of malaria plasmodium, that interest was awakened in the subject. Golgi demonstrated that the small, hyaline, intracellular rings and the crescents were often associated with fevers of remittent character with long intervals between

the paroxysms, but he did not actually describe these forms as belonging to a distinct species or name the species.

Marchiafava and Celli (1889) and Canalis (1889) were the first to accurately describe this plasmodium and to separate it into a distinct species. These observers, working independently, traced the entire life-cycle of this plasmodium in the blood of man, and demonstrated that the organism differed from *Plasmodium vivax* and *Plasmodium malariae* in its morphology, life-cycle in man, and in the clinical symptoms that it produced in man. But little has been added to their descriptions of the plasmodium, and, although errors of interpretation are present in their descriptions, they stand today as accurate records of the morphology of *Plasmodium falciparum*.

The specific name "*falciparum*" was given to the plasmodium by Welch, in 1897, and is accepted as the proper specific name of this species by the best authorities. The generic name "*Plasmodium*," until recently almost universally accepted, has been replaced by the generic name "*Laverania*" by some recent writers, but I see no good reason for adopting this name, and I believe that the name *Plasmodium falciparum*, which has become fixed in the nomenclature, should be retained. This subject is more fully discussed on page 362.

**Morphology.**—The morphology of *Plasmodium falciparum* will be described as the parasite appears in both unstained and stained preparations of blood, for it is essential that the student should be able to recognize the plasmodium in either the living or stained condition. Unlike *Plasmodium vivax* and *Plasmodium malariae*, the pigmented forms, or *schizonts*, are found only rarely in the peripheral blood, the hyaline "ring-forms," or *trophozoites*, being the only forms observed in the peripheral blood, in ordinary infections, besides the crescentic *gametes*. In pernicious infections young pigmented forms may often be observed, and sporulating forms may also be present in very small numbers, but, in the vast majority of infections with *Plasmodium falciparum*, the sporulating forms are found only in the capillaries of the internal organs, especially in the spleen, bone marrow, and brain, and pigmented forms are very rarely observed in the peripheral blood.

**Morphology of *Plasmodium falciparum* in Unstained Preparations.**—In unstained preparations of blood *Plasmodium falciparum*, in its earliest intracorpuscular stage of development, is noted within or upon the infected red blood corpuscle as a hyaline "ring" or disk, from 2 to 3 microns in diameter, well defined and sluggishly motile, the periphery of the plasmodium undulating and sending forth minute pseudopodia at irregular intervals. By reason of the amoeboid motion the young "ring-forms," or *trophozoites*, frequently become disk-like in shape. The rings are somewhat irregular in shape, one portion of the ring being considerably broader



than the remainder, thus giving rise to the so-called "signet-ring" form, an appearance practically never observed in the quotidian æstivo-autumnal "rings." Infection of the erythrocyte with more than one plasmodium occurs, but not so frequently as with the quotidian æstivo-autumnal plasmodium, and it is rather rare to find more than two plasmodia in a corpuscle, while in the quotidian subspecies three, and even four, plasmodia are frequently observed within the infected erythrocyte. The ring-forms of this species are frequently as large as the ring-forms of *Plasmodium vivax*, but are distinguished from those of the latter species by the thickening at one portion of the periphery which causes the "signet-ring" appearance, and by the thicker appearance of the ring due to the larger amount of cytoplasm, the ring-form of *Plasmodium vivax* being thin and delicate in appearance. As a rule it is only these ring-forms that are observed in the peripheral blood in ordinary instances of infection with *Plasmodium falciparum*, the pigmented rings and other forms, about to be described, occurring only in the internal organs.

The ring-forms gradually increase in size until, at the end of from sixteen to eighteen hours, they measure as much as 3.5 microns in diameter, and at this time a few fine grains of reddish-brown or almost black pigment may be observed within them, generally lying in the enlarged area of the ring. The pigment is apparently sluggishly motile, the motility, as in all malaria plasmodia, being due to cytoplasmic currents within the body of the parasite. After the development of pigment the ring-form is soon lost, the plasmodium increases in size, becomes more clearly defined, the cytoplasm appearing very refractive and slightly granular under very high magnifications. As the parasite develops the pigment tends to collect in a more or less solid mass, at or near the centre of the organism. After the development of pigment the amœboid motility of the parasite is retained for several hours, but is sluggish in character and gradually disappears. The bizarre-shaped plasmodia so common in infections with *Plasmodium vivax* are very rarely observed, but I have seen forms similar to the forms described as *Plasmodium tenue*, by Stephens, in some infections with *Plasmodium falciparum*, and these forms resemble closely the amœboid stage of *Plasmodium vivax*, in which the irregular and bizarre types of organism are most frequently observed.

In the usual infection with *Plasmodium falciparum* only the ring-forms and a very few of the pigmented ring-forms are observed in the peripheral blood, the larger pigmented forms occurring in the peripheral blood only in very severe or pernicious infections, although a very careful and prolonged examination of the blood may show, even in infections of average severity, a very few half- or three-quarter grown pigmented *schizonts*.

When fully developed, just prior to sporulation, the pigmented

*schizonts* of *Plasmodium falciparum* occupy from two-thirds to three-quarters of the infected erythrocyte and sometimes *schizonts* are observed that practically fill the infected corpuscle. Sporulation occurs approximately every 48 hours in uncomplicated infections, but may be delayed as long as 50 hours or occur as early as 44 to 46 hours, but it never occurs in 24 hours, as claimed by those who believe in only one species or variety of the æstivo-autumnal plasmodia. At the time of sporulation the plasmodium fills from two-thirds to almost the entire erythrocyte, the pigment, of an almost black color, being collected in a solid, oval, or spherical mass at or near the centre. The *merozoites*, or spores, appear as hyaline, oval, refractive bodies, collected about the mass of pigment in a more or less regular manner. It is difficult to determine their number in unstained preparations, but careful examinations have convinced me that they may vary in number from 10 to 30, the average number varying between 18 and 24. Sporulation occurs within the erythrocyte, but not infrequently the infected corpuscle is so entirely filled with the sporulating organism that very little of it can be observed. The sporulating forms occur very rarely in the peripheral blood, and only in very severe or pernicious infections, but blood obtained by splenic puncture will contain many of these forms.

The infected red blood corpuscle, in infections with *Plasmodium falciparum*, is never enlarged, as in infections with the benign tertian plasmodium, *Plasmodium vivax*, but is usually slightly smaller than the normal red blood corpuscles, and darker green in color. Crenation of the infected corpuscles is frequently observed, especially in those cells containing nearly full-grown or sporulating parasites, or in cells containing more than one plasmodium, but the wrinkled, shrunken appearance, so frequently observed in erythrocytes infected with the quotidian æstivo-autumnal plasmodium, is never observed, in my experience.

Bass (1920) states that in cultures *Plasmodium falciparum* develops to almost the diameter of the infected red blood corpuscle, and often produces 24 or more *merozoites*. He also calls attention to the fact that the ring-form is frequently as large as that of *Plasmodium vivax*, and may be considerably thicker and heavier in appearance. In my experience the ring-forms of *Plasmodium falciparum*, in the vast majority of instances, contain a larger amount of cytoplasm than do those of *Plasmodium vivax*, so that in examining specimens of blood containing only the ring-forms one is very apt to believe them to be benign tertian plasmodia rather than æstivo-autumnal, owing to the almost universal, although erroneous teaching, that the ring-forms of *Plasmodium vivax* are larger than those of *Plasmodium falciparum*.

The *gametes*, or sporogenic forms of *Plasmodium falciparum* and *Plasmodium falciparum quotidianum*, which are intended to undergo development in the mosquito, are distinguished from the *gametes* of other

species of malaria plasmodia by their crescentic shape, and hence are generally called "crescents" in the common nomenclature of malaria. These *gametes*, or crescents, occur only in the blood of individuals suffering from infection with the æstivo-autumnal plasmodia, and only after the infection has existed for at least two weeks, according to the best observations. The *gametes* are very resistant to quinine, and persist in the peripheral blood long after the *schizonts* have disappeared, although continued treatment with quinine will eventually result in their disappearance.

In unstained preparations of blood the *gametes* appear at first as hyaline, oval, or spherical bodies which are distinguished from the ring-forms, or *schizonts*, by lack of amoeboid motility and the absence of the ring-like appearance. When pigment develops in these young *gametes* it is greater in amount and darker brown in color, and the cytoplasm of the parasite is more granular in appearance and more refractive. As growth increases the pigment also increases in amount, is immotile, and generally arranged in a more or less irregular manner throughout the cytoplasm. The shape of the *gamete* within the red blood corpuscle tends to become more and more crescentic, as development occurs, and when fully grown and ready to be liberated from the corpuscle the shape is definitely crescentic. At this time the erythrocyte in which the *gamete* has developed has shrunk about the parasite, forming a membrane-like covering, one portion of which, connecting the poles of the crescentic *gamete*, forms a hemispherical projection called the "bib" of the crescent. In the youngest crescentic forms the pigment is distributed throughout the cytoplasm, but in the older forms it becomes collected at the centre or toward one of the extremities. When fully developed, the crescent, or *gamete*, appears free in the blood plasma, the border being represented by a single or double refractive outline, often of a bright-green color, while the so-called "bib" is plainly visible as a lighter-green hemispherical mass situated in the concavity of the crescent. The sex of the crescents, or *gametes*, is easily ascertained in infections with *Plasmodium falciparum*, the male *gamete* being shaped like a plump kidney bean, while the female *gamete* is more typically crescentic, being long and slender in appearance.

The *microgametocyte*, or male crescent or *gamete*, is much more plump in appearance than the female crescent, or *macrogametocyte*, and the shape is more like that of a lima bean or kidney bean than a typical crescent. These forms measure from 7 to 10 microns in length by 3 to 5 microns in breadth, and may have a double or single border of a greenish color. The cytoplasm appears less granular and opaque than the cytoplasm of the *macrogametocyte*, and the pigment occurs in the form of fine granules distributed throughout the cytoplasm, and may occur even at the poles of the crescent. As in the case of the *microgametocytes* of the other malaria plasmodia, exflagellation may sometimes be observed in these crescents in



fresh preparations, the *microgametocyte* becoming ovoid and finally almost spherical in shape, while the pigment becomes violently agitated, and eventually several slender filaments are projected from the periphery of the parasite and lash about among the red blood corpuscles. These filaments are the *microgametes*, and soon become free in the blood plasma. The process of exflagellation occurs normally in the mid-gut of the mosquito.

The *macrogametocyte*, or female *gamete* or crescent, is distinguished by its slender crescentic form, and is longer and more narrow than the *microgametocyte*, measuring from 10 to 15 microns in length by 2 to 3 microns in breadth. The ends of the *gamete* are sharply rounded or even pointed, in the *macrogametocyte*, while in the *microgametocyte* the ends of the organism are bluntly rounded. The *macrogametocyte* is surrounded by a single or double greenish border and the pigment is dark brown in color, immotile, and either concentrated in the centre of the crescent in a dense mass or arranged in a wreath-like manner around the centre. Although the pigment is often collected in what appears to be a single mass at the centre of the crescent, careful examination shows that the pigment granules are not fused, but can be separately distinguished, and this is also true of the pigment when it is collected in a wreath-like formation. The cytoplasm is opaque, very refractive, and finely granular in appearance. After the *macrogametocyte* becomes extracellular, which normally occurs in the mid-gut or stomach of the mosquito but which often occurs in the blood if it be collected upon a moistened slide, it becomes ovoid and then spherical in shape, having a clear-cut border, and the pigment arranged in a wreath-like manner about the centre of the parasite. Not infrequently the pigment, in these forms, is divided into small, spherical, almost black dots, arranged in a perfect circle surrounding the central area of the crescent. These forms are really *macrogametes*, having undergone maturation phenomena which have prepared them for fertilization by the *microgametes*.

#### Morphology of *Plasmodium falciparum* in Stained Preparations.—

The staining reactions of *Plasmodium falciparum* with Wright's stain, or other modifications of the Romanowsky stain, are similar to those of the other malaria plasmodia, the cytoplasm of the parasite staining blue, the chromatin red, while the nutritive vacuole and achromatic zone remain unstained.

The *trophozoites*, or young ring-forms, consist of a ring of blue stained cytoplasm, surrounding a large unstained nutritive vacuole, at one side of which is situated a small milky-appearing vesicle which contains a dot of ruby-red chromatin, the nucleus. Instead of a single dot of chromatin two dots may be present, either close together or situated at opposite sides of the "ring." The comparative thickness of the ring-form



of this species is well shown in stained preparations, and many of the rings present a marked enlargement, generally situated directly opposite the chromatin dot which represents the nucleus.

The *trophozoites*, or young ring-forms, are often observed applied to the edge of the red blood corpuscle, and this appearance is quite

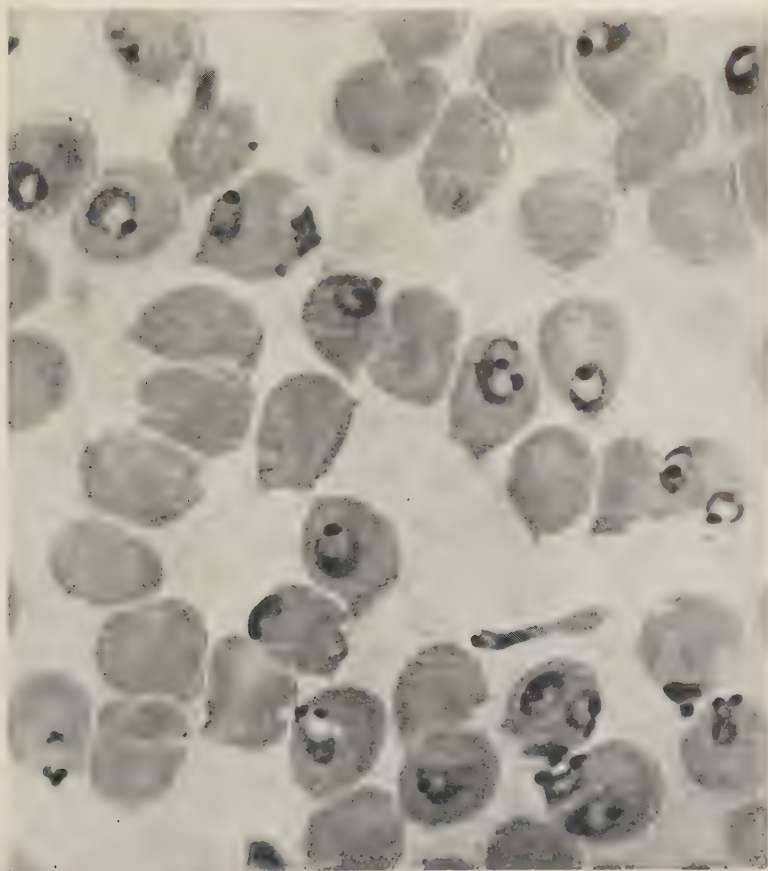


FIG. 74.—Photomicrograph of "ring-forms" of *Plasmodium falciparum* in blood of a fatal case of æstivo-autumnal malaria. Note the enlargement of the "ring" opposite the dot of chromatin, which is characteristic of the "ring-forms" of this species of plasmodium.  $\times 2,000$  Wright's stain. (Army Medical School Collection.)

characteristic of infections with *Plasmodium falciparum*. Such parasites appear as a slender lengthened thread or spindle of blue-stained cytoplasm containing a red chromatin dot either at one end or somewhere along the length of the blue-stained cytoplasm, the entire parasite lying on a portion of the periphery of the erythrocyte.

In some of the ring-forms of this species pigment may be observed lying in the expanded portion of the ring and appearing as greenish brown,

minute granules in stained preparations. Pigmented rings can usually be found in stained preparations if carefully searched for, even in mild infections with *Plasmodium falciparum*, but pigmented ring-forms are never observed in infections with the quotidian variety of æstivo-autumnal plasmodia, *Plasmodium falciparum quotidianum*.

In stained preparations the ring-forms of *Plasmodium falciparum* vary from 2 to 3 microns in diameter, but may, in isolated instances, measure as little as 1.5 and as much as 3.5 microns in diameter. They are characterized by their large size, as compared with the quotidian subspecies, the larger amount of cytoplasm, which is expanded at some portion of the periphery of the ring-form, in the vast majority of plasmodia, and by the frequent presence of pigment grains in the expanded portion of the ring.

The larger pigmented plasmodia of this species, the *schizonts*, present in stained preparations a blue-stained cytoplasm in which may be observed a small amount of chromatin in the form of fine grains collected within an unstained vesicular area representing the nucleus. The pre-sporulating plasmodia consist of a mass of blue-stained cytoplasm in which lie small irregular clumps of ruby-red chromatin and a small amount of greenish-black pigment collected in an irregular mass near the centre of the plasmodium.

In stained preparations the sporulating forms of *Plasmodium falciparum* almost fill the infected erythrocyte and measure from 5 to 6 microns in diameter. The spores, or *merozoites*, are arranged more or less regularly around a mass of greenish pigment, each *merozoite* consisting of a small blue-stained mass of cytoplasm containing a spherical ruby-red or violet dot of chromatin. The *merozoites* can be easily counted and vary in number, in my experience, from 10 to 30, the average running between 18 and 24.

In stained preparations the infected erythrocyte may or may not appear crenated, and stains a pink or salmon-pink color. A few basophilic granules may be observed in some of the infected cells, which are known as "Maurer's dots," and which are easily differentiated from "Schüffner's dots," so characteristic of infections with *Plasmodium vivax*. The infected red blood corpuscles are usually normal in size and shape, although a few may be slightly smaller than normal and more or less distorted in shape. However, reduction in size of the infected cell is not common in infections with this plasmodium.

The staining reactions of the *gametes* of *Plasmodium falciparum* are essentially similar to those of the benign tertian and the quartan *gametes*, when Wright's stain is employed, the cytoplasm staining blue, the chromatin red or violet, while the milky zone surrounding the chromatin is absent in these forms.

In their earliest stage of development the *gametes* are distinguished

from the *trophozoites* by the situation of the chromatin dot *within* the centre of the ring-like parasite instead of at some portion of the periphery, there being no signet-ring appearance of the plasmodium which is so characteristic of the *trophozoites* of this species of plasmodium. The youngest *gametes* consist of a uniform ring of blue-stained cytoplasm containing at the centre a red dot of chromatin. As the *gamete* develops the blue-stained cytoplasm is larger in amount and assumes a crescentic shape, while the chromatin consists of grains of ruby-red or violet material collected at the centre or distributed in the cytoplasm. After development has reached the crescentic stage it is possible to easily identify the male and female *gametes* by their staining reactions.

The *microgametocyte*, or male crescent or *gamete*, stains poorly, the cytoplasm staining a delicate blue or pale-green color, in many instances being almost colorless. The chromatin stains a delicate pink or red, and is in the form of a loose network extending over the greater portion of the crescent, the poles usually being free from chromatin. The pigment appears granular and greenish-black in color, and is distributed throughout the cytoplasm, being intermingled with the chromatin, but the grains of pigment are distinct. The chromatin is often stained poorly or so finely divided that it is seen with difficulty in some of the *microgametocytes*. In those organisms in which the *microgametes* are being formed, which are oval or round in shape instead of crescentic, the cytoplasm stains somewhat more intensely, and the chromatin is collected into irregular masses, stained a deep red, at the periphery of the plasmodium. When exflagellation has occurred the body of the *microgametocyte* consists largely of red-stained chromatin, and the *microgametes* may be observed projecting from it as delicate pink or reddish undulating fibrils, apparently consisting almost entirely of chromatin.

The *microgametocytes* may be distinguished from the *macrogametocytes* in stained preparations by attention to the following points:

1. The plump kidney shape of the crescent or *microgametocyte*.
2. The pale blue or greenish staining of the cytoplasm.
3. The arrangement of the pale red chromatin in a loose network, occupying a large portion of the cytoplasm.
4. The distribution of the pigment throughout the cytoplasm. The remains of the red blood corpuscle, which surround the *microgametocyte*, stain a salmon- or bright-pink color, and not infrequently a deep-red band may be observed closely enveloping the crescent, especially in those crescents which are apparently free in the blood plasma. This deeply stained border, or band, undoubtedly represents the capsule which surrounds the *gametes*.

The pink-stained remainder of the red blood corpuscle in which the

*microgametocyte* has developed often appears as an irregular, jagged rim around the entire crescent, and the so-called "bib" as a faintly staining

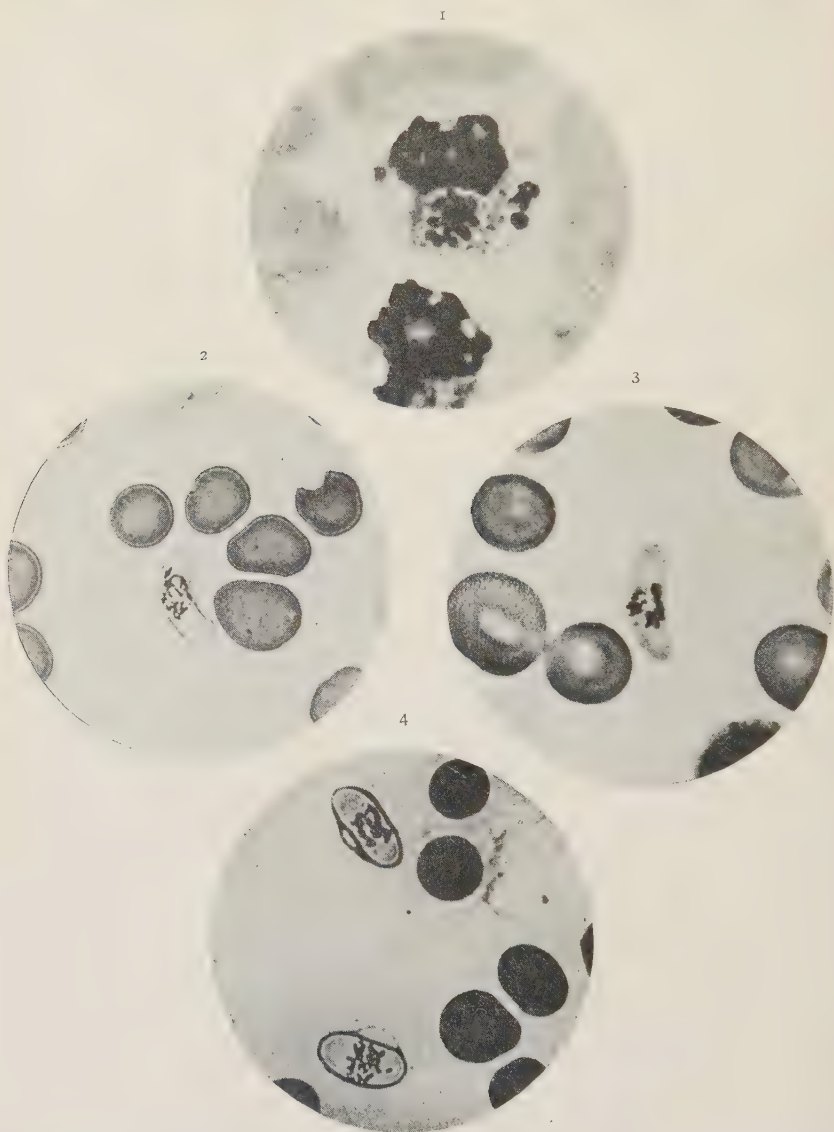


FIG. 75.—*Plasmodium falciparum*. Tertian æstivo-autumnal plasmodium. (Photomicrographs. Army Medical School Collection.) Wright's stain. 1. Large mononuclear leucocyte, or macrophage, containing malarial pigment and a sporulating form of *Plasmodium falciparum*.  $\times 1,800$ . 2. A macrogametocyte of *Plasmodium falciparum*.  $\times 1,200$ . 3. A macrogametocyte of *Plasmodium falciparum*.  $\times 1,500$ . 4. Two microgametocytes of *Plasmodium falciparum*.  $\times 1,200$ . These are the so-called crescents, the macrogametocyte being the female gamete, and the microgametocyte the male gamete.

hemispherical mass occupying the concavity of the crescent, having a brightly stained, pink border.



The *macrogametocyte* stains much more intensely than does the *microgametocyte*, the cytoplasm being colored a deep blue, more marked at the poles of the crescent than in the middle. The shape of the *macrogametocyte* is more crescentic, being longer and more slender than the *microgametocyte*, while the ends are almost pointed or sharply rounded. The chromatin stains a bright ruby red or violet, and is collected at or near the centre of the crescent in larger granules and in irregular particles which often appear fused together. The pigment is greenish brown in color, and often surrounds the chromatin as a distinct wreath or is collected in small masses near the chromatin instead of being distributed throughout the cytoplasm as in the *microgametocyte*.

In stained preparations the *macrogametocyte*, or female crescent or *gamete*, may be distinguished from the *microgametocyte*, or male crescent, by the following morphological features:

1. The long slender shape of the crescent.
2. The situation of the chromatin in a more or less dense mass at or near the centre of the crescent.
3. The deep-blue staining of the cytoplasm.
4. The concentration of the pigment in small masses or in a wreath-like manner about the chromatin.

The staining reactions of the red blood corpuscles in which the *macrogametocytes* have developed are similar to those already described in the cells infected by the *microgametocyte*.

As already noted, *gametes* or crescents do not occur in every infection with *Plasmodium falciparum*, and only in infections that have lasted for a period of at least twelve to fourteen days. In my experience only about 50 per cent. of infections with this plasmodium show *gametes* in the blood and in regions where the infections are recognized promptly and promptly and properly treated, the percentage of infections showing crescents is very much lower. As will be noted in the discussion of the prophylaxis of infection with the malaria plasmodia, it is possible to prevent the formation of *gametes* in practically 100 per cent. of infections by prompt diagnosis and proper treatment.

**Habitat.**—*Plasmodium falciparum* is a parasite of the erythrocytes of man and, so far as is definitely known, does not occur in any of the lower animals. However, as shown by Reichenow, Blacklock and Adler, and Adler, chimpanzees and gorillas are infected with a plasmodium that is

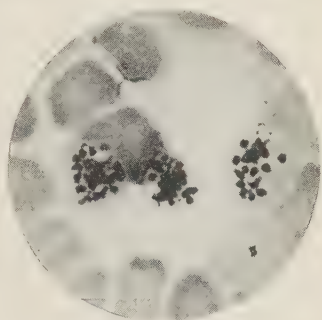


FIG. 76.—*Plasmodium falciparum*.  
X 1,200. Three sporulating forms of  
*Plasmodium falciparum*. (Photomicrograph. Army Medical School Collection.)

morphologically indistinguishable from *Plasmodium falciparum*, and further observations may prove that the parasites are identical.

**Species Occurring in Lower Animals.**—Reichenow (1920), in the Cameroons, found plasmodia indistinguishable morphologically from *Plasmodium falciparum* in the blood of 5 out of 8 chimpanzees which he examined, two showing the crescentic *gametes* alone, one *Plasmodium falciparum* and *Plasmodium vivax* forms, and two *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium malarie* forms. Blacklock and Adler (1922) found a plasmodium resembling *Plasmodium falciparum* in the blood of a chimpanzee. Both *schizonts* and crescentic *gametes* were found, but were accompanied by forms resembling *Plasmodium vivax* and *Plasmodium malarie*. Adler (1923) examined 13 chimpanzees, from the Sierra Leone Protectorate, and found two infected with a plasmodium indistinguishable morphologically from *Plasmodium falciparum*. Both *schizonts* and crescentic *gametes* were found, but forms resembling either *Plasmodium vivax* or *Plasmodium malarie* were not detected. Post-mortem, the organs of these animals were found pigmented, especially the spleen, liver, and bone marrow, and the plasmodia were found in smears from these organs and from the bone marrow.

Although the plasmodia found by the above investigators in the blood of chimpanzees are morphologically similar to *Plasmodium falciparum*, there is no evidence that they are really identical with this species. Attempts to infect man with plasmodia from the chimpanzee have proven unsuccessful in the hands of Blacklock and Adler (1922) and attempts to infect the chimpanzee by Blacklock and Adler, by the injection of human blood infected with *Plasmodium falciparum*, have likewise given negative results.

As stated above, plasmodia similar in morphology to *Plasmodium falciparum* occur in the blood of the chimpanzee, and some authorities, as Reichenow, believe that this species is a parasite of the anthropoid apes, but more work remains to be done before it can be stated that *Plasmodium falciparum* occurs in these animals. Other species of plasmodia which occur in the blood of the lower animals have already been mentioned. (See page 389.)

**Cultivation.**—*Plasmodium falciparum* was first cultivated *in vitro* by Bass and Johns (1912) in the culture medium composed of human blood to which a certain percentage of dextrose had been added. In cultures the entire human life-cycle of the plasmodium may be followed, and the morphology is similar, in both fresh and stained preparations, to the forms observed in the blood of man. This plasmodium has been cultivated by many investigators, following the method of Bass, so that his observations have been amply confirmed.

**Life-history.**—The life-history of *Plasmodium falciparum* is similar

to that of the other malaria plasmodia, the human cycle of existence, or *schizogony*, being passed within the red blood corpuscles of man, while the mosquito cycle of existence, or *sporogony*, is passed within the body of mosquitoes belonging to the *Anophelinae*. The life-cycle in man is completed in from 40 to 48 hours, the *schizont* dividing into from 10 to 30 *merozoites*, which invade new corpuscles, and repeat the cycle; while the life-cycle in the mosquito is completed in from 10 to 12 days, at the end of which time the salivary glands contain *sporozoites* which infect man when the insect bites.

**Geographical Distribution.**—The geographical distribution of *Plasmodium falciparum* is practically confined to subtropical and tropical regions, and it is a rare species in temperate localities. The distribution of this species is world-wide, and in the tropics it furnishes the great bulk of malarial infections, although it may be much more numerous in some localities than in others. Thus, at Camp Stotsenberg, in the Philippine Islands, there occurred from three to four cases of infection with *Plasmodium falciparum* to one of infection with *Plasmodium vivax*, the benign tertian plasmodium, but at Camp Gregg, about fifty miles from Camp Stotsenberg, the prevailing type of infection was the benign tertian.

The geographical distribution of *Plasmodium falciparum* may be said to be that of pernicious malaria, for wherever fatal malarial infections occur there we find infections with this plasmodium, and a very large proportion of the fatal infections are due to this species. Owing to this fact the geographical distribution of *Plasmodium falciparum*, as well as of the quotidian variety, is of great interest to American practitioners, for infections with these plasmodia occur not only in the southern portions of the United States, but are very prevalent in our colonial possessions, the Philippine Islands, Porto Rico, and in the Canal Zone. Severe infections with *Plasmodium falciparum* occur in the Gulf States and in the Mississippi Valley especially, and also in the valleys of the Sacramento and San Joaquin rivers in California. Almost all of the islands of the West Indies are infested with this plasmodium, and Cuba is badly infested in certain localities. The coast regions of Mexico and Central America are badly infested, and the deadly Chagres fever, of Panama, is caused by this species

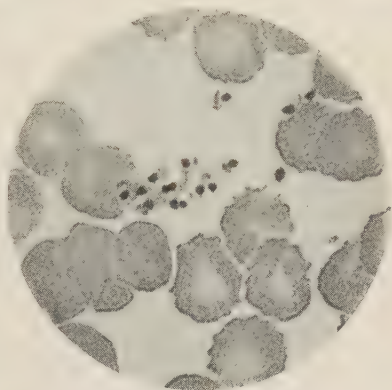


FIG. 77.—*Plasmodium falciparum*. Tertian æstivo-autumnal plasmodium. (Photomicrograph. Army Medical School Collection.) Free spores, or merozoites of *Plasmodium falciparum*.  $\times 1,500$ .



of plasmodium. *Plasmodium falciparum* is the cause of pernicious malaria in most of the coast States of South America, and in Italy, Greece, Crete, Sicily, and Turkey infections with this plasmodium are common and often fatal. The coast regions of southern Asia and of Africa are badly infested with this plasmodium, and the river valleys of most of the countries in southern Asia are endemic centres of infection with this species. In all of these regions *Plasmodium falciparum* is the cause of many fatal infections. The valleys of the Senegal, Congo, and Niger rivers, in Africa, are among the most dangerous lurking places of this plasmodium, as well as the regions around the great lakes and the jungles and lake shores of Abyssinia. In Formosa and the Philippines infections with this species are common, and in the Philippines such infections are very fatal in certain localities.

The continent of Australia is free from infection with this or other species of malaria plasmodia, and this is also true of most of the islands of Polynesia, including the Hawaiian Islands.

**Incidence of Infection.**—The incidence of infection with *Plasmodium falciparum* varies greatly in different localities, the species being practically absent in many parts of the temperate zones, while in some localities in the tropics and subtropics it causes from 50 to 80 per cent. of the malarial infections encountered. It is the common species of æstivo-autumnal plasmodium, the quotidian variety being very rare in comparison.

The incidence of infection with this plasmodium in the affected portions of the United States varies greatly, in some regions only a few cases of infection with it being observed during the malarial season, while in others the vast majority of infections are due to this plasmodium. It may be stated that in the Gulf States, in the infected regions of the Mississippi Valley, and in the Sacramento and San Joaquin valleys in California, from 50 to 60 per cent. of malarial infections are due to *Plasmodium falciparum*, although in all of these regions localities occur in which the incidence of infection with this species is much lower.

After the Spanish-American War infection with *Plasmodium falciparum* was introduced into some of the Northern States, where it had never been hitherto observed, and in these instances the incidence of infection was sometimes high. In an outbreak in a city in Connecticut, which I observed, and which was caused by soldiers returning there from service at Santiago, Cuba, practically 80 per cent. of the cases of malaria observed during the summer and fall months were caused by this plasmodium.

In most localities in the subtropics, and especially in the tropics, where malaria is endemic, the incidence of infection with *Plasmodium falciparum* is much higher than with either *Plasmodium vivax* or *Plasmodium*



*malaria*. In the examination of the blood of over 2,000 soldiers returning from the Philippine Islands and infected with malaria, I found no less than 1,662, or 80.3 per cent., infected with *Plasmodium falciparum*, and at Camp Stotsenberg, on the island of Luzon, I found that 75 per cent. of the malarial infections were caused by this plasmodium. In Italy and Greece, as shown by Grassi, Marchiafava and Bignami, Celli, Cardamantis, and others, infections with *Plasmodium falciparum* are much more numerous than infections with the other malaria plasmodia, and this statement is generally true of all regions in the tropics and subtropics where severe and fatal malarial infections occur.

In some regions, however, where infections with *Plasmodium falciparum* occur, the percentage of incidence is lower. Muhlen (1913) found that in 2,071 malarial infections which he observed at Jerusalem, 988, or only 47.7 per cent., were due to *Plasmodium falciparum*, and Leger and Baury (1922), in Dakar, found that 47 per cent. of the malarial infections among the natives were due to this plasmodium. A still lower percentage of incidence with this species has been noted in many tropical regions, but only in certain limited localities, but as the temperate zone is approached the incidence of infection with this plasmodium markedly decreases, and *Plasmodium vivax* becomes the predominant species. As will be noted in the discussion of latent malarial infection, a very large proportion of apparently healthy natives in regions infested with *Plasmodium falciparum* are infected with this plasmodium.

**Method of Transmission.**—*Plasmodium falciparum* is transmitted to man, and from man to man, by mosquitoes belonging to the *Anophelinae*, and the first positive results that were obtained in the transmission of malaria by the bites of infected anophelines were obtained with mosquitoes that had bitten patients suffering from infection with this plasmodium. Bignami (1898) was the first to prove that the mosquito transmitted this plasmodium by producing a typical attack of the fever in a healthy individual by the bites of mosquitoes that had fed upon a patient having *Plasmodium falciparum* in his blood, and his results have since been confirmed by numerous observers, so that today no one questions the transmission of this, as well as of all species of plasmodia, by the mosquito. Only mosquitoes belonging to the *Anophelinae* are able to transmit *Plasmodium falciparum*, and many species belonging to this family have been found capable of transmitting the parasite. The following table gives the species of *Anopheles* that have been proven to transmit this species, with the name of the observer and the place and date of the observation. It will be noted that no less than 36 species of *Anopheles* are capable of transmitting *Plasmodium falciparum* to man.

*Mosquitoes Transmitting Plasmodium falciparum, with Name of Observer, Place, and Date*

Species of Mosquito	Observer	Place	Date
<i>A. aconitus.</i>	James and Staunton.	Malay States.	1902
<i>A. albimanus.</i>	Darling.	Panama.	1909
<i>A. annulipes.</i>	Kinoshita.	Formosa.	1906
<i>A. argyrotarsis.</i>	G. de Faria.	Brazil.	1910
<i>A. barbirostris.</i>	Walker and Barber.	Malay States.	1911
<i>A. bifurcatus.</i>	Grassi.	Europe.	1898
<i>A. costalis.</i>	Ross, Annett, Austin.	Africa.	1899
<i>A. crucians.</i>	King, Mitzmain.	Louisiana.	1916
<i>A. culicifacies.</i>	Stephens and Christophers.	India.	1902
<i>A. formosaensis, II</i>	Tsuzuki.	Formosa.	1902
<i>A. fuliginosus.</i>	Stephens and Christophers.	India.	1902
<i>A. fumestus.</i>	Daniels.	Africa.	1901
<i>A. hunteri.</i>	Barber.	Malay States.	1918
<i>A. indefinitus.</i>	Barber.	Malay States.	1918
<i>A. intermedius.</i>	Ladislao and Neiva.	Brazil.	1918
<i>A. kawari.</i>	Barber.	Malay States.	1918
<i>A. kochi.</i>	Barber.	Malay States.	1918
<i>A. lindesayi.</i>	Stanton.	Malay States.	1914
<i>A. listoni.</i>	James.	India.	1902
<i>A. ludlowi.</i>	Banks.	Philippines.	1907
<i>A. maculipalpis.</i>	Stephens and Christophers.	India.	1902
<i>A. maculipennis.</i>	Grassi, Bignami.	Europe.	1898
<i>A. minimus.</i>	Tsuzuki.	Formosa.	1902
<i>A. pharoensis.</i>	Newstead, Dutton, Todd.	Africa.	1907
<i>A. punctipennis.</i>	King, Mitzmain.	Louisiana.	1916
<i>A. pseudopunctipennis.</i>	Darling.	Panama.	1910
<i>A. pulcherrimus.</i>	James.	India.	1902
<i>A. quadrimaculatus.</i>	Thayer.	United States.	1900
<i>A. rossi.</i>	Stephens and Christophers.	India.	1902
<i>A. sinensis.</i>	Grassi.	Italy.	1898
<i>A. stephensi.</i>	Stephens and Christophers.	India.	1902
<i>A. tarsimaculata.</i>	Darling.	Panama.	1909
<i>A. tessalatus.</i>	Shüffner.	Java.	1918
<i>A. theobaldi.</i>	Stephens and Christophers.	India.	1902
<i>A. turkhudi.</i>	Stephens and Christophers.	India.	1902
<i>A. umbrosus.</i>	Barber.	Malay States.	1918

While the 36 species of *Anopheles* mentioned in the table have been found infected with *Plasmodium falciparum* either in nature or experimentally, it should be remembered that many of these species do not act in nature as common transmitters of the plasmodium, and, so far as the United States is concerned, the only species of great importance in this respect are *A. quadrimaculatus*, *A. punctipennis*, and *A. crucians*, while in the Canal Zone *A. albimanus* is the most important of the mosquitoes transmitting *Plasmodium falciparum*.

**Experimental Infection of Lower Animals.**—Many attempts have been made to experimentally infect lower animals with *Plasmodium falciparum*, but all have been unsuccessful. The fact that the logical experimental animals, *i.e.*, the higher apes, are infected with plasmodia that are practically indistinguishable morphologically from *Plasmodium falciparum*

renders their use questionable in experiments along this line, and there is no reason to believe that any other of the lower animals are susceptible to infection with the human malaria plasmodia. The plasmodium that has been studied by Reichenow (1920), Blacklock and Adler (1923), and Adler (1924), that occurs in chimpanzees, is very similar in morphology to *Plasmodium falciparum*, but the fact that these investigators have proven that man is not susceptible to this plasmodium, while the chimpanzee cannot be infected with *Plasmodium falciparum*, demonstrates apparently that the two parasites are distinct, and Adler (1924) has proposed the name *Plasmodium reichenowi* for the plasmodium of the chimpanzee.

**Relation to Disease.**—*Plasmodium falciparum* is the cause of a common and characteristic form of malarial

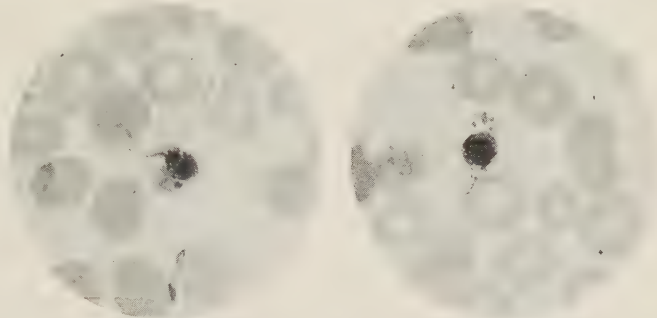


FIG. 78.—*Plasmodium falciparum*. Two flagellated microgametocytes of *Plasmodium falciparum*. (Photomicrograph. Army Medical School Collection.)  $\times 1,200$ .

infection which is known as æstivo-autumnal tertian fever, or malignant tertian fever. That the plasmodium is the cause of the disease has been experimentally proven both by the direct inoculation of blood containing the plasmodia into man and by the bites of Anopheline mosquitoes which had been infected by allowing the insects to bite individuals whose blood contained the *gametes* of this species of plasmodium.

Such experiments have practically always resulted in the production of the clinical symptoms of tertian æstivo-autumnal malaria, and the typical plasmodium has appeared in the blood after a definite period of incubation. This species was first proven to be the cause of the disease by the direct inoculation of blood containing the plasmodium into healthy individuals, and the following table gives the results obtained in several such experiments, together with the period of incubation and the name of the investigator.

Observer	Plasmodium Inoculated	Period of Incubation	Type of Fever
Bignami.	<i>Plasmodium falciparum</i> .	6 days.	Æs., Tertian.
Bignami.	<i>Plasmodium falciparum</i> .	10 days.	Æs., Tertian.
Bastianelli and Bignami.	<i>Plasmodium falciparum</i> .	3 days.	Æs., Tertian.
Bastianelli and Bignami.	<i>Plasmodium falciparum</i> .	4 days.	Æs., Tertian.
Bastianelli and Bignami.	<i>Plasmodium falciparum</i> .	5 days.	Æs., Tertian.
Bastianelli and Bignami.	<i>Plasmodium falciparum</i> .	4 days.	Æs., Tertian.
Panichi.	<i>Plasmodium falciparum</i> .	5 days.	Æs., Tertian.

From this table it will be observed that the *incubation period* of *Plasmodium falciparum* after direct inoculation varied between 3 and 10 days, but that most of the experiments gave a period of incubation between 4 and 5 days, and that the average period of incubation was practically 5 days. This period of incubation is much shorter than after infection by the bites of mosquitoes, either experimentally or after natural infection, as will be seen by the results recorded regarding the period of incubation after the experimental production of infection with *Plasmodium falciparum* by the bites of infected mosquitoes, which follow:

Observer	Type of Mosquito Infection	Period of Incubation	Type of Fever
Schüffner.	<i>Plasmodium falciparum</i> .	15 days.	Æs., Tertian.
Jancso.	<i>Plasmodium falciparum</i> .	10 days.	Æs., Tertian.
Jancso.	<i>Plasmodium falciparum</i> .	10 days.	Æs., Tertian.
Jancso.	<i>Plasmodium falciparum</i> .	14 days.	Æs., Tertian.
Jancso.	<i>Plasmodium falciparum</i> .	11 days.	Æs., Tertian.
Jancso.	<i>Plasmodium falciparum</i> .	13 days.	Æs., Tertian.
Bastianelli and Bignami.	<i>Plasmodium falciparum</i> .	12 days.	Æs., Tertian.

The above table shows that the shortest period of incubation after the bites of mosquitoes infected with *Plasmodium falciparum* was 10 days, and the longest 15 days, the average period of incubation in these experiments being nearly 12 days. It may, therefore, be stated that numerous experiments have shown that the period of incubation of *Plasmodium falciparum* after the bite of infected mosquitoes is between 10 to 12 days, in the vast majority of cases in which successful results have been obtained, a period twice as long as after the direct inoculation of human blood containing the parasite.

Under natural conditions the period of incubation, in the vast majority of cases, agrees roughly with that after the bite of infected mosquitoes experimentally, it having been repeatedly observed that troops entering regions where *Plasmodium falciparum* is endemic begin to show cases of infection in from 10 to 12 days, and though longer periods of incubation are frequently observed it may be stated that the period of incubation after natural infection generally closely agrees with that after the experimental production of infection by the transmitting mosquitoes.

Marchiafava and Bignami (1900) observed three cases of infection with this plasmodium in which they could be sure of the period of incubation. The first case was that of a young man living in the central part of Rome, where malaria did not occur. He visited a very malarial town near the Pontine marshes, remained overnight, being bitten by many mosquitoes, and returned to Rome. Nine days after his return he developed æstivo-autumnal fever, and *Plasmodium falciparum* was found in his blood.

The second case was that of an engineer, living in Rome, who had



never suffered from malaria, and who was obliged to spend a day in a place in the Pontine marshes, where he was badly bitten by mosquitoes. At the end of *ten* days he developed an æstivo-autumnal infection, and *Plasmodium falciparum* was found in his blood.

The third case was that of a lady who was exposed to the bites of malarial mosquitoes and who developed æstivo-autumnal tertian fever after an incubation period of from *nine* to *ten* days.

Jackson (1905) describes a most interesting instance in which a limited epidemic of malaria occurred in the members of a troop of United States cavalry, under circumstances in which the period of incubation could be accurately determined. Of 45 soldiers belonging to a troop of the 6th U. S. Cavalry, exposed at the same time and under similar conditions to infection with *Plasmodium falciparum*, no less than 18 developed tertian æstivo-autumnal fever, the incubation period in all varying between *ten* and *eleven* days. Other authorities, as Ziemann, Mannaberg, and Navarre, have recorded instances in which the period of incubation of this plasmodium after natural infection varied between 10 and 12 days, thus agreeing with the period observed after experimental infection by anopheline mosquitoes.

However, it should be remembered that the period of incubation in this, as in all other species of malaria plasmodia, varies greatly under natural conditions, and instances have been observed in which it was greatly prolonged in infections with *Plasmodium falciparum*. The following instances, which I observed personally, well illustrate the great variations in the period of incubation that are sometimes observed.

In August, 1889, a surgeon of the United States Army was stationed in a malarious locality in Cuba, remaining at this station until September, when he returned to New York, and was then ordered to San Francisco. While in Cuba he had no symptoms of malarial infection, and after reaching New York he went immediately to San Francisco. After reaching that city he remained well until March, 1900, but during that month suffered from malaise and diarrhœal attacks. On April 1st he had a slight chill, and his temperature rose to 106.2° F. An examination of his blood showed numerous hyaline and pigmented forms of *Plasmodium falciparum*. In this instance the infection was undoubtedly acquired in Cuba, as æstivo-autumnal malaria was not present in either New York or San Francisco, and he was in no other places than these after leaving Cuba. The incubation period in this case was at least *seven months*. Had he remained in Cuba he would probably have developed symptoms much earlier, but the change to a more favorable climate and the consequent benefit to his general health undoubtedly delayed the period of incubation.

In another instance two officers of the United States Army, stationed at a malarious post in the Philippine Islands, never suffered from malaria

while there, but after returning to the United States, one developed an attack of malaria in one month and the other in four months after his return.

Many more instances could be cited of long periods of incubation after infection with *Plasmodium falciparum*, and some authorities have cited instances in which the period of incubation was from one to two years, but such cases must be very rare. I have never observed a period of incubation longer than seven months, and that in one case only. It is undoubtedly true that in these cases an examination of the blood would probably show the presence of *Plasmodium falciparum* for a considerable period before the occurrence of symptoms, and I have followed such cases for days, and even weeks, and observed the plasmodia in their blood, although no symptoms of malaria were present. In these cases the long periods of incubation were due to the small number of plasmodia present and to the natural resistance of the individuals to infection and the toxic effects of the plasmodia.

While *Plasmodium falciparum* is the cause of tertian æstivo-autumnal malarial fever, there are many instances observed in which infection with this parasite does not result in the production of noticeable symptoms of malarial fever. An examination of the blood of the natives in any endemic malarial area will show that a very considerable proportion of both children and adults show the plasmodium in their blood in small numbers, but no definite symptoms of the infection are present. These individuals often present *gametes*, or crescents, in their blood, and are of great importance as carriers of the infection, and will be more fully considered in the discussion of the prophylaxis of the malaria plasmodia. The immunity that these individuals possess to the toxins produced by the plasmodia has been acquired through repeated attacks of malaria, generally in early youth, so that they may harbor the plasmodia for years without any severe symptoms of malaria being evident.

## Subspecies II. *PLASMODIUM FALCIPARUM* QUOTIDIANUM, Craig, 1909.

Synonyms: *Plasmodium immaculatum*, Grassi and Feletti, 1890. Quotidian æstivo-autumnal plasmodium, Marchiafava and Bignami, 1891. Pigmented quotidian æstivo-autumnal plasmodium, Mannaberg, 1893. Unpigmented quotidian æstivo-autumnal plasmodium, Mannaberg, 1893. *Plasmodium quotidianum*, Bates, 1913.

**History and Nomenclature.**—*Plasmodium falciparum* *quotidianum* was first described, in part, by Grassi and Feletti (1890), who evidently saw it, but confused it with *Plasmodium falciparum*, for parts of their description of *Plasmodium immaculatum*, as they called it, apply to both of these plasmodia. Marchiafava and Bignami (1891–1892) were the first to separate this plasmodium from *Plasmodium falciparum*, calling it the

quotidian æstivo-autumnal plasmodium. Their description was accurate, and they called attention to the fact that the quotidian species differed from the tertian æstivo-autumnal species in the length of the cycle of development in man, 24 hours instead of 48 hours, and in its smaller size, and lesser amœboid activity. Marchiafava and Bignami regarded the quotidian plasmodium as a distinct species of æstivo-autumnal plasmodium, but they did not give it a specific name. Mannaberg (1893) recognized three species of æstivo-autumnal plasmodia, the malignant tertian and a pigmented and unpigmented quotidian plasmodium, but he did not give specific names to these parasites. Mannaberg undoubtedly saw and described the quotidian plasmodium described by Marchiafava and Bignami, but his observations regarding an unpigmented quotidian plasmodium have not been confirmed, although Ziemann described a pernicious type of plasmodium occurring in Africa as not forming any pigment.

My own observation upon the plasmodia concerned in the etiology of the malarial fevers early convinced me that more than one parasite was concerned in the etiology of the æstivo-autumnal fevers, that a type of malarial fever occurred characterized by quotidian paroxysms which could not be due to double infections with *Plasmodium vivax* or triple infections with *Plasmodium malariae*, and that associated with this type was a distinctive form of the æstivo-autumnal plasmodium which could be distinguished from *Plasmodium falciparum* both morphologically and clinically. This conclusion was arrived at before I had the opportunity of seeing Marchiafava and Bignami's contribution, but a perusal of their work convinced me that the plasmodium that I had been studying was identical with the quotidian æstivo-autumnal plasmodium described by them. Later, in 1909, believing that this plasmodium should be regarded as a subspecies of *Plasmodium falciparum*, I named it *Plasmodium falciparum quotidianum*.

**Morphology of *Plasmodium falciparum* *quotidianum* in Unstained Preparations.**—*Plasmodium falciparum* *quotidianum* is first noted in the infected erythrocyte as a very minute hyaline ring, generally a little over 0.5 micron in diameter, though frequently plasmodia are observed that measure less than this in diameter. The very minute size of the ring-forms of this plasmodium in their earliest stage of development in the blood corpuscles of man is an important differential point, and it is undoubtedly true that the parasite is generally overlooked at this time unless one is an expert in the examination of blood for malaria plasmodia, owing to the minuteness of the rings.

At first the outline of the ring-form is indistinct, but it soon becomes well defined and amœboid motility develops, the periphery of the ring sending out minute pseudopodia at irregular intervals, the motion being so rapid that only a very careful examination will detect it. This fact

explains why Marchiafava and Bignami considered that the quotidian plasmodium was less amœboid than the tertian æstivo-autumnal plasmodium, for there is really little, if any, difference between the two parasites in this respect. The typical ring-form is often lost during the periods of amœboid activity, the plasmodium appearing triangular or disk-like in shape, but amœboid activity is not constant, and for long periods of time may be absent, the typical ring-form being retained.

In from two to four hours the ring-forms increase in size to about one micron in diameter, and become more distinct and refractive. The outline of the ring is very sharply cut in the erythrocyte, looking as though it had been punched into the corpuscle, but the ring-form is more slender than that of *Plasmodium falciparum*, owing to the smaller amount of cytoplasm present, and the "signet-ring" appearance, so common in the ring-forms of the latter species, is not observed in the ring-forms of *Plasmodium falciparum quotidianum*, the ring appearing of the same thickness throughout, and consisting of a thin ring of cytoplasm surrounding a minute spherical area of the same color as the infected corpuscle. At this time amœboid activity is not so marked as in the earlier stages of development and is often entirely absent. I have never observed pigment in a ring-form of this species, but in infections with *Plasmodium falciparum* the ring-forms are frequently pigmented. Double and triple infections of the erythrocyte frequently occur, and in fatal pernicious cases some of the red blood corpuscles may contain as many as five or six of the ring-forms.

When pigmentation occurs the ring-form is lost, the plasmodium appearing as a hyaline, oval, or spherical disk containing one or two rather coarse granules of dark-brown or almost black pigment, situated at the periphery or near the centre, and perfectly motionless. At this stage of development the organism seldom fills more than one-fifth of the infected erythrocyte, which is shrunken and crenated in appearance and of a dark olive-green color.

After the appearance of pigment the plasmodium gradually increases in size until at the end of twenty-two to twenty-four hours it fills from one-third to one-half of the infected corpuscle. The pigment is collected in a very small solid block, spherical, or irregular in shape, at or near the centre of the parasite, which is well defined and refractive. Amœboid activity has ceased and evidences of sporulation are noted, consisting of delicate striations dividing the plasmodium into several minute spores or *merozoites*. Owing to their very minute size, generally less than 0.5 microns in diameter, it is very difficult, and often impossible, to distinguish the number of *merozoites* in unstained preparations, but in stained preparations they are found to number from 6 to 18, the average being from 12 to 14, as shown by my counts of many hundreds of the sporulating *schizonts*.

In *Plasmodium falciparum quotidianum* sporulation occurs at the end



of 24 hours, and I have never observed a case of infection with this plasmodium in which it was delayed for more than an hour or two, or occurred earlier than 22 hours. In uncomplicated cases of infection sporulation always occurs every 24 hours, thus differentiating it from *Plasmodium falciparum*, in which sporulation occurs every 48 hours in most instances, and always later than 36 hours.

At the time of sporulation the plasmodium fills about one-half of the infected erythrocyte, in the vast majority of infections, but sometimes not more than one-third of the infected cell is occupied by the parasite. I have never observed quotidian plasmodia practically filling the erythrocyte, as is common in infections with *Plasmodium falciparum*. At this stage of development the infected erythrocyte is generally considerably distorted in shape, shrunken in appearance, of a dark olive-green color, and often the hæmoglobin appears retracted about the plasmodium. The pigmented and sporulating forms of *Plasmodium falciparum quotidianum* do not usually occur in the peripheral blood, but in severe and pernicious infections such forms are generally present in considerable numbers in blood obtained from the lobe of the ear or the finger. However, in most instances, only the ring-forms will be found in the peripheral blood, as is the case with infections with *Plasmodium falciparum*.

In infections with the quotidian æstivo-autumnal plasmodium the invaded red blood corpuscle always presents marked morphological changes, which are caused by the growth and development of the plasmodium within it. These changes are much more marked than in infections with *Plasmodium falciparum*, and are best studied in unstained preparations. They consist in a marked reduction in size, a crenated and shrunken appearance of the cell, considerable distortion in shape, and a change in color to a very dark olive-green or "brassy" appearance. The so-called "brassy" bodies, *i.e.*, infected erythrocytes, presenting a shrunken appearance, and of a brass-like color, are more frequently observed in infections with this plasmodium than with *Plasmodium falciparum*, but they are not characteristic of infection with the quotidian variety.

The gametes of *Plasmodium falciparum quotidianum* are crescentic in shape and closely resemble those of *Plasmodium falciparum*, except that they are only a little over half as long, and both the male and female are more plump in appearance. In unstained preparations the male, or *microgametocyte*, is very short and broad, resembling a lima bean in shape, and is generally surrounded by a double outline, resembling a capsule, which is greenish in color. The female, or *macrogametocyte*, is more slender, but is much more plump than the *macrogametocyte* of *Plasmodium falciparum*, and is also surrounded by a greenish appearing, double outline, resembling a capsule. The pigment in both male and female gametes is very small in amount, dark brown or almost black in color, and in the form of granules

and minute rod-like bodies which are collected toward the centre of the *gamete* or at one of the poles.

Bass (1920) states that in cultures the quotidian æstivo-autumnal plasmodium produces about 16 *merozoites* at the time of sporulation, and that the sporulating plasmodia occupy about one-half or slightly more of the infected erythrocyte, while the sporulating forms of *Plasmodium falciparum*, the tertian æstivo-autumnal plasmodium, fill almost the entire red blood corpuscle. He also calls attention to the very small ring-forms, the ring-forms of the tertian æstivo-autumnal species being much larger. The morphology of *Plasmodium falciparum quotidianum* in cultures is like that observed in the blood of man, both in unstained and stained preparations.

It is very difficult to differentiate the ring-forms of *Plasmodium falciparum quotidianum* from those of *Plasmodium falciparum* in unstained preparations unless one has had considerable practice in the examination of blood for the malaria plasmodia, as the differentiation depends entirely upon the smaller size of the quotidian rings and their more sharply-cut outline within the infected erythrocyte. For this reason many infections with this species are overlooked if only unstained preparations are studied, and it is always advisable to use stained preparations in the examination of blood from patients suffering from æstivo-autumnal infections, as the ring-forms are much more easily recognized in such preparations, and it is possible to differentiate the species of plasmodia with greater ease and certainty.

**Morphology of *Plasmodium falciparum quotidianum* in Stained Preparations.**—In stained preparations of blood *Plasmodium falciparum quotidianum* may be readily distinguished from *Plasmodium falciparum* by one trained in the study of the malaria plasmodia. The ring-forms are characterized by the small amount of cytoplasm and the relatively large amount of nuclear chromatin, while the larger forms are distinguished by their small size and the smaller number of *merozoites* in the sporulating plasmodia. With Wright's or other modifications of the Romanowsky stain, the cytoplasm of the plasmodium stains blue, while the nuclear chromatin stains a bright-red or violet color. The vesicular portion of the nucleus is unstained.

The smallest intracorpuseular forms of this subspecies, or the *trophozoites*, are so very minute that they may be overlooked, in stained preparations, even by those who are more or less expert in examining malarial blood. They measure as little as 0.5 micron, or even less, in diameter, the smallest consisting of a minute granular dot of chromatin, stained pink or red in color, enclosed in a very minute amount of cytoplasm, stained blue, but so small in quantity that it requires the most careful examination to detect it. In this stage of development the plasmodium may be mistaken

for a very minute blood-plate or mere cellular detritus, as the true ring-form is not present, and it is undoubtedly true that at this very early stage of intracorpuseular development the plasmodium is very frequently overlooked or mistaken for something else.

At a slightly later stage of development the true "ring-form" is present, but is often so very minute that a very careful examination is necessary in order to demonstrate it. In these very minute ring-forms the cytoplasm is represented by a delicate blue-stained ring which contains somewhere along its circumference an irregular, semi-lunar mass of red- or violet-stained chromatin. The amount of chromatin is very large as compared with the amount of cytoplasm, and ring-forms are frequently observed in which the chromatin mass comprises one-half or even more of the ring, and sometimes almost the entire plasmodium appears to be composed of chromatin.

In this subspecies the chromatin is not arranged in the form of a spherical dot or dots at some portion of the circumference of the ring, but usually occurs as a semi-lunar shaped mass forming a portion of the actual ring, in some instances almost the entire ring being formed of one or more of these semi-lunar masses of chromatin, practically no cytoplasm being visible. The centre of the ring, *i.e.*, the portion of the cytoplasm of the red blood corpuscle enclosed by the ring-form, is absolutely colorless, thus indicating that this portion of the parasite is composed of a substance that does not take the stain and which also prevents the substance of the red blood corpuscle from staining, for the centre of the ring-forms of the other species of plasmodia show the eosin-staining reaction of the red corpuscle. The appearance described is very characteristic of the ring-forms of *Plasmodium falciparum quotidianum* in stained preparations, the ring-forms of *Plasmodium falciparum* presenting an entirely different appearance, the centre of the ring staining pink or salmon, as does the remainder of the infected red blood corpuscle. The very definite white, unstained centre of the ring-forms of *Plasmodium falciparum quotidianum* causes the plasmodia to appear as though stamped through the substance of the infected erythrocyte with a punch, and in pernicious infections with this subspecies almost every infected erythrocyte will present the colorless spot surrounded by the cytoplasm and chromatin of the tiny plasmodium, and when multiple infection of the red blood corpuscle occurs, as is frequently observed, the invaded cells present a very characteristic appearance, looking as though filled with holes, each surrounded by the cytoplasm and chromatin of a parasite. The fully developed ring-forms do not show the unstained central portion described, the enclosed portion of the infected cell staining pink or salmon color.

The fully developed ring-forms, or those which have attained their largest size, present a greater amount of blue-stained cytoplasm, but even

in these forms the relative amount of chromatin to cytoplasm is much greater than in *Plasmodium falciparum*, and the chromatin appears as irregular ragged masses at some portion of the periphery of the ring or as a large semi-lunar-shaped mass or masses forming a considerable portion of the ring. As in the smaller ring-forms, some of the full-grown rings appear to be composed almost entirely of chromatin, due to the large size of the semi-lunar mass or the merging into one another of two such masses.

This peculiar richness of the ring-forms of the quotidian subspecies in chromatin, and its arrangement within the ring, is a most important

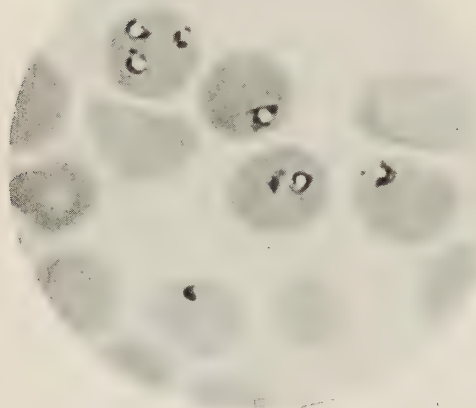


FIG. 79.—*Plasmodium falciparum* quotidianum. Photomicrograph. (From Army Medical School Collection.)  $\times 1,800$ . Note minute size of the "rings" and the peculiar arrangement of the chromatin (deeply stained portion) in a semi-lunar mass forming a large part of the cytoplasm of the "ring." Also clear area within ring resembling a vacuole and more sharply defined than in *Plasmodium falciparum*.

differential feature, for in the ring-forms of *Plasmodium falciparum*, the subtertian species, the chromatin is very small in amount in comparison with the amount of cytoplasm, and is arranged in the form of one or two perfectly spherical dots at some portion of the periphery of the ring, and never in irregular clumps or semi-lunar masses, which comprise a large portion of the ring, as in the quotidian subspecies.

The pigmented and pre-sporulating forms of *Plasmodium falciparum* contain a comparatively small amount of blue-stained cytoplasm and a relatively large amount of pink- or red-stained chromatin, arranged in the form of irregular granular clumps or threads scattered throughout the cytoplasm. Pigmented ring-forms are never observed, as in *Plasmodium falciparum*, the ring-form always being lost before pigmentation occurs. In the pre-sporulating plasmodia the chromatin is arranged irregularly throughout the cytoplasm, while the pigment, green or black in color, is collected in a compact, small spherical or irregular mass near or at the centre of the parasite. The pigmented and pre-sporulating forms are small, seldom exceeding 3 or 4 microns in diameter, and generally measure about 2 to 2.5 microns in diameter, almost always filling less than one-half of the infected erythrocyte.

The sporulating forms of *Plasmodium falciparum* quotidianum in stained preparations measure from 2.5 to 3 microns in diameter, in the vast majority of instances, but smaller and larger forms may be rarely



observed in some infections. They fill about one-half of the infected red blood corpuscle, or a little more in many instances, and appear to be composed mostly of chromatin, owing to the fact that the *merozoites* are composed very largely of this material. The plasmodia at this stage of development are seen to consist of numerous oval or spherical collections of very finely granular chromatin, stained a pink or reddish violet, each collection embedded in a very minute mass of blue-stained cytoplasm. The pigment appears almost black in color and is collected in a solid block or irregular clump at or near the centre of the sporulating parasite.

When sporulation is complete the *merozoites*, or spores, appear as distinct oval or round, very minute bodies, stained a dark red or violet, and surrounded by a minute amount of blue-stained cytoplasm. They are very small, not measuring more than one-half micron in diameter in stained preparations, and it is often very difficult to determine their exact morphology, owing to their minute size. They are characterized by the relatively large amount of chromatin, the *merozoites* generally appearing to be composed almost entirely of this substance, and this fact differentiates them from the *merozoites* of *Plasmodium falciparum*, in which the chromatin is limited to a small spherical dot or mass situated in a relatively large amount of chromatin.

The number of *merozoites*, or spores, varies considerably in different plasmodia, but in the counts that I have made of many hundred sporulating plasmodia of this subspecies, I have never observed more than 18 *merozoites* nor less than 6, the average running between 12 and 14, the latter number being most frequently encountered in individual plasmodia. As already stated, the sporulating plasmodia rarely fill more than one-half of the infected red blood corpuscle, while the sporulating forms of *Plasmodium falciparum* practically fill the entire infected cell in the majority of instances, and as many as 24 *merozoites* may be commonly observed.

In stained preparations the erythrocytes infected with *Plasmodium falciparum quotidianum* are almost always distorted in shape, and appear smaller, in many instances, than the uninfected corpuscles. The cytoplasm

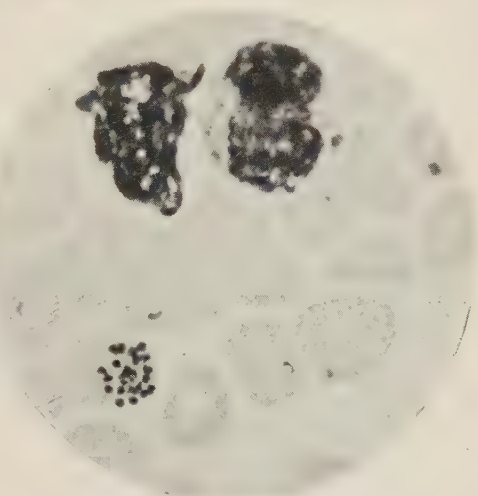


FIG. 80.—*Plasmodium falciparum quotidianum*.  $\times 1,600$ . (Photomicrograph. Army Medical School Collection.) A sporulating form and two of the minute "ring-forms." Note that the sporulating form fills only about half of the infected red blood corpuscle.

always stains poorly, and basophilic granulation is frequently observed in the infected corpuscle. The characteristic unstained portion of the cell surrounded by the ring-form of the plasmodium has already been noted, and is probably due to a vacuole or to the vesicular portion of the nucleus of the ring-form. It constitutes one of the most important differential points in the diagnosis of the quotidian plasmodium at this early stage of development, as it is never observed in the cells infected with the ring-forms of the tertian æstivo-autumnal plasmodium. In the latter the substance of the infected erythrocyte within the ring-form may stain poorly, but there is never the absolute lack of color and the "hole-like" appearance that is noted with infections with the quotidian subspecies.

Double, triple, and quadruple infections of the erythrocyte with the ring-forms of the quotidian subspecies are frequently observed, and double infections are very common. Owing to their distorted shape, it is frequently impossible to determine whether the infected erythrocytes are reduced in size, but in cells that have preserved their shape it is noted that there is a considerable reduction in size, as compared with the uninfected cells surrounding them.

The *gametes* of *Plasmodium falciparum quotidianum* are identical in morphology with those of *Plasmodium falciparum*, being crescentic in shape when fully developed, but are only about one-half the size of the latter species, and much more plump in appearance, both the *microgametocyte* and the *macrogametocyte* being shaped like a kidney bean rather than like a typical crescent. They present the same staining reactions as the *gametes* of *Plasmodium falciparum*, but the young intracellular forms are distinguished from the latter by the much greater relative amount of chromatin, resembling in this the ring-forms of the *schizonts*.

In most infections with *Plasmodium falciparum quotidianum* only the unpigmented ring-forms and the crescentic *gametes* are found in the peripheral blood, but in many severe infections pigmented ring-forms may be found with little trouble, and even some larger pigmented forms. In infections developing pernicious symptoms the larger pigmented forms and sporulating forms can usually be found in the peripheral blood, if the examination is patiently and carefully made, and it is certainly true that such forms occur much more frequently in the blood in infections with the quotidian subspecies than in infections with *Plasmodium falciparum*. In my experience all pernicious infections with this plasmodium show pigmented and sporulating plasmodia in the peripheral blood if the patient dies of the infection.

**Habitat.**—*Plasmodium falciparum quotidianum* is a parasite of the red blood corpuscles of man, in which it undergoes its human life-cycle, or *schizogony*. It is also a parasite of certain species of anopheline mosquitoes, in which it undergoes its mosquito life-cycle, or *sporogony*. In

man the plasmodium lives within the erythrocytes, and develops at the expense of these cells. That it does not live and develop upon the surface of the red blood corpuscle, as believed by Rowley-Lawson, is proven by the peculiar structure of the ring-forms already described. The development of the plasmodium in the mosquito has not, as yet, been described by any authority, but it is probable that it undergoes the same development in this insect as do the other species of malaria plasmodia.

**Species Occurring in Lower Animals.**—No observations have been reported that indicate that *Plasmodium falciparum quotidianum* ever occurs in any of the lower animals, or that any species of plasmodium closely resembling it is a parasite of lower animals. While several investigators have reported the occurrence of a plasmodium apparently identical with *Plasmodium falciparum* in chimpanzees, none of the plasmodia so far observed in the blood of these animals resemble *Plasmodium falciparum quotidianum*, so far as the descriptions indicate.

**Cultivation.**—This plasmodium was cultivated by Bass (1912), who states that the forms observed in cultures resemble those observed in the blood of man, and that no development of the *gametes* occurs in the cultures. The medium used for cultivating the plasmodium was the dextrose-blood medium used by Bass in cultivating the other species of malaria plasmodia. (See Appendix.)

**Life-history.**—*Plasmodium falciparum quotidianum*, like the other malaria plasmodia, has two life-cycles, one completed in the blood of man and the other in the mosquito. The human life-cycle, or *schizogony*, is completed in man in approximately 24 hours, but we are yet ignorant of the length of time consumed by the parasite in its development in the mosquito, owing to the lack of experimental data regarding the *sporogony* of this parasite. We have no record of the morphology of the forms of this parasite found in the mosquito, but there is no reason to believe that they differ in any essential respect from the developmental forms found in the mosquito during the *sporogony* of the other malaria plasmodia, especially those of the closely related species, *Plasmodium falciparum*. In man the parasite sporulates approximately every 24 hours, as already stated, the malarial paroxysm coinciding with the time of sporulation.

**Geographical Distribution.**—The geographical distribution of *Plasmodium falciparum quotidianum* is apparently much more limited than that of *Plasmodium falciparum*, the tertian æstivo-autumnal plasmodium, and owing to the fact that the quotidian parasite is very rare, and that the ring-forms are so frequently overlooked, or confused with those of *Plasmodium falciparum*, the exact geographical distribution of this subspecies has not been ascertained. I have observed cases of infection with *Plasmodium falciparum quotidianum* that originated in Cuba, Panama, the Philippine Islands, and in the southern part of the United States, but

there are many localities in all of these countries in which this parasite is unknown, although *Plasmodium falciparum* may be a common species in such regions. Thus at Camp Stotsenberg, in the Philippine Islands, both the tertian and quotidian types of æstivo-autumnal plasmodia were present, although the quotidian parasite was rarely found, but at Camp McKinley, some sixty miles from Camp Stotsenberg, the quotidian plasmodium was never encountered, although *Plasmodium falciparum* was frequently encountered.

In some localities *Plasmodium falciparum quotidianum* is excessively rare. Thus, in many hundreds of blood-smears from scores of malarial patients in Panama and the Canal Zone that I have examined I have found this plasmodium in but two cases, and this fact undoubtedly explains why this species has been overlooked by investigators in the Canal Zone.

It cannot be said that at the present time the exact geographical distribution of this parasite has been ascertained, but it undoubtedly occurs in certain localities in Cuba, Panama, the Philippine Islands, and the southern, or Gulf State, portion of the United States.

**Incidence of Infection.**—We possess very little data regarding the incidence of infection with *Plasmodium falciparum quotidianum* in localities in which this subspecies occurs, but what we have is sufficient to prove that it is very rare, as compared with either *Plasmodium vivax* or *Plasmodium falciparum*, and that just as infections with *Plasmodium malarie*, the quartan plasmodium, are rare, as compared with infections with the benign tertian plasmodium, *Plasmodium vivax*, so are infections with the quotidian æstivo-autumnal plasmodium as compared with infections with the tertian æstivo-autumnal plasmodium, or *Plasmodium falciparum*. In 1,611 æstivo-autumnal infections that I have personally studied, 1,473 were caused by *Plasmodium falciparum*, and only 189 by *Plasmodium falciparum quotidianum*, and these infections were all observed in patients who contracted their infections in localities where both parasites were present.

In most localities in which *Plasmodium falciparum* occurs the quotidian subspecies is apparently absent or so rare as to be overlooked or confused with the former plasmodium. From personal observation I know that in some regions where *Plasmodium falciparum* is a common parasite *Plasmodium falciparum quotidianum* is absent, while in other localities it is very rare. So far as known, there is no locality where the quotidian subspecies is the prevailing type, or where it occurs alone, but wherever it does occur it is only infrequently encountered.

The comparative rarity of this plasmodium is one of the chief reasons for its not having been generally recognized as a distinct plasmodium, just as the rarity of the quartan plasmodium (*Plasmodium malarie*) caused it



to be overlooked for long periods of time in various localities. For instance, during the early years of work upon the Panama Canal it was repeatedly officially reported that the quartan plasmodium did not occur in the Canal Zone, but later it was found that, while rare, as it is in most localities, it was present but had remained unrecognized. The same condition prevails today in many localities as regards the recognition of the quotidian æstivo-autumnal plasmodium, and if one remembers the exceedingly minute size of the "ring-forms" of this subspecies, which are the forms usually noted in the peripheral blood, and the infrequency of infections with this parasite, I think that it is evident why so many observers in malarial regions have failed to note its presence, or have not recognized it, when present.

**Method of Transmission.**—*Plasmodium falciparum quotidianum* is undoubtedly transmitted from man to man by anopheline mosquitoes, but the exact species of anopheles concerned has not been ascertained. We have no records of the experimental production of infection of man with this subspecies by the bites of an infected mosquito, so that the period of incubation after the bite of such an insect has not been determined. The rarity of infections with this plasmodium indicates that special conditions are necessary for its transmission, but what these special conditions are has not been determined, although it is probable that they have to do with the species of mosquito involved and their relation to man.

**Experimental Infection of Lower Animals.**—So far as recorded, no attempts have been made to infect any of the lower animals with *Plasmodium falciparum quotidianum*.

**Relation to Disease.**—The constant presence of this plasmodium in the blood of patients suffering from a type of malarial fever characterized by a quotidian temperature, and the absence of the plasmodium from the blood of patients suffering from other types of malarial fever, is sufficient proof of the etiological relationship of the plasmodium to the disease. The only experimental data that we possess as to the relation of the plasmodium to disease are furnished by the observations of Gerhardt (1884), who produced typical quotidian æstivo-autumnal malarial fever in two individuals by the direct inoculation of blood containing *Plasmodium falciparum quotidianum* obtained from a patient suffering from a malarial fever characterized by quotidian paroxysms. In one of the experimentally produced infections the incubation period was 7 days, and in the other the incubation period was 12 days. A great deal more experimental work should be done upon the question of the species of mosquitoes concerned in the transmission of this plasmodium, the exact period of incubation of the plasmodium in the mosquito before the insect becomes infective, and the experimental production of the infection in man.

## DOUBTFUL SPECIES OF MALARIA PLASMODIA

If one consults the synonyms of the accepted species of malaria plasmodia it is evident that numerous observers have, from time to time, described plasmodia which they believed to be new species, but which have proven to be identical with well-known species, but Stephens has described a plasmodium which has distinct claims to specific status, *i.e.*, *Plasmodium tenue*, a parasite observed in the blood of man in the Central Provinces, in India.

## PLASMODIUM TENUE, Stephens, 1914

**History and Nomenclature.**—In two identical contributions published separately, Stephens (1914) described a plasmodium which he studied in a blood-slide sent him by Major Kenrick, I. M. S., from Pachmari, Central Provinces, India, and which he believed to be a new species. Both of the contributions were illustrated with the same drawings, and demonstrated conclusively that the plasmodia were all in practically the same stage of development, so that the species was described from only one stage of development, and that an early stage before the production of pigment. This plasmodium was named by Stephens *Plasmodium tenue*, because of the peculiar tenuous appearance of the amœboid forms of the organism. He stated that he believed that it had affinities with the simple tertian plasmodium and with *Plasmodium canis*, of the dog. Stephens considered that it differed from *Plasmodium falciparum* in having greater amœboid activity and in the abundance and irregularity of the nuclear chromatin, and from *Plasmodium vivax* in being much smaller, having more delicate amœboid processes, a larger amount of, and a more marked irregularity in the distribution of the chromatin, and in the rarity of the typical “ring-form.”

In a contribution (1914) in which I discussed *Plasmodium tenue*, I stated: “From the description of this organism given by Stephens, and the drawings which accompany the description, it is, to say the least, extremely doubtful if this parasite can be accepted as a new species of malaria plasmodia. Even though it differed from the known species to a much greater extent than is described, it would be entirely unjustifiable to describe it as a new species from the morphology of the parasite during such a small portion of the life-cycle and from the organisms observed in a single stained blood-smear.” As I had observed plasmodia similar in morphology to *Plasmodium tenue* in blood from tertian infections I considered, at that time, that it was probably an atypical variant of *Plasmodium vivax*, perhaps produced by the administration of small doses of quinine, as it is well known that this drug will stimulate amœboid activity in such doses, and the forms that I had observed and which resembled

*Plasmodium tenue* had all occurred in the blood of patients upon small doses of quinine.

Recently, Sinton (1922) has published a very important paper describing *Plasmodium tenue* as observed in five cases occurring in the Central Provinces, India, within 100 miles of the region in which Kenrick (1914) secured the blood-smear upon which Stephens made his observations. Sinton has been able to study the entire life-cycle of the plasmodium in man, and his observations apparently demonstrate that it should be classified among the plasmodia causing æstivo-autumnal malaria, as crescentic gametes were found to be present. Sinton is of the opinion that *Plasmodium tenue* is distinct from *Plasmodium falciparum*, and that it is entitled to be recognized as a separate species provided that a "tenue" phase does not exist in the life-cycle of the latter plasmodium.

As regards the occurrence of the typical "tenue" forms in the ordinary cycle of *Plasmodium falciparum* in the blood of man, I am sure, from personal observations, that such forms do not occur, although I have seen forms closely resembling them in the blood of patients infected with this plasmodium who have taken quinine; but no quinine was administered in the case in which Sinton made his observation upon morphology, so that in this instance the "tenue" forms could not have been caused by the drug.

In his contribution Sinton calls attention to the marked resemblance of *Plasmodium tenue* to *Plasmodium immaculatum*, Grassi and Feletti, as pictured in the atlas of Meyer and Rieder (1907) and states: "I have no doubt that the two parasites are identical." If they are, the name *Plasmodium tenue* will have to be abandoned, and the organism should be called *Plasmodium immaculatum* by law of priority. However, *Plasmodium immaculatum* was described by Grassi and Feletti as having a quotidian periodicity and as being unpigmented, so far as the forms encountered in the peripheral blood were concerned, whereas *Plasmodium tenue* has a tertian periodicity, and pigmented forms are numerous in the peripheral blood. It is my opinion that *Plasmodium immaculatum* is probably identical with *Plasmodium falciparum quotidianum*, and that the illustrations of Meyer and Rieder depicting very amœboid forms were drawn from forms occurring in the blood of patients who had taken quinine, for Grassi and Feletti do not mention such marked amœboid forms as characteristic of *Plasmodium immaculatum*, as they appear to be of *Plasmodium tenue*.

Sinton's observations go a long way toward establishing *Plasmodium tenue* as a distinct species, and it may be stated that at the present time the evidence is pretty conclusive that this plasmodium is entitled to specific rank.

**Morphology in Stained Preparations.**—The following description of the morphology of *Plasmodium tenue* is based upon the descriptions of

Stephens (1914) and Sinton (1922). All of their observations were made upon stained blood-smears, and there is no description of the appearance of this parasite in fresh blood preparations available.

The youngest forms of *Plasmodium tenue* observed in the peripheral blood are very small, round, or slightly oval "rings," varying in size from 1.5 to 2.25 microns in diameter, the cytoplasm staining blue and forming a very delicate ring slightly thickened opposite the dot of red-stained chromatin, which is situated at the periphery of the plasmodium, but which does not project beyond the border of the "ring" to the degree that it does in the ring-forms of *Plasmodium falciparum*. At a little later stage of development the ring-forms are more oval in shape, and some of them are drawn out into a tail-like projection, giving a tadpole-like appearance to the parasite. The chromatin dot is sometimes oval in outline, and two such dots may be present. The unstained centre of the "ring" or vacuole is very well marked, resembling that of *Plasmodium falciparum quotidianum*, so far as can be judged by the descriptions and illustrations. The infected red blood corpuscle is distorted in shape, and double infections of the corpuscle are not uncommon.

After from 10 to 12 hours the plasmodia have increased in size, measuring from 2.5 to 3 microns in diameter and occupying from one-third to two-fifths of the infected corpuscle. Various forms are observed at this time, the most common being large oval or tailed forms, while irregular amœboid and oval forms with blunt projections are sometimes observed. The vacuole is distinct, and the chromatin irregular, occurring as oval and band-like masses, which may be double or treble in number. The chromatin may be situated within the vacuole or forming a band or semicircle around its edge, an appearance frequently observed in the ring-forms of *Plasmodium falciparum quotidianum*, as noted in the description of that organism. Pigment is not present in these forms, but the infected red blood corpuscle shows some evidence of basophilic degeneration.

In from 26 to 32 hours the true "tenue" forms are found, consisting of the most bizarre amœboid plasmodia, the pseudopodia of which are most delicate in consistence and stain poorly. These forms resemble the amœboid forms of *Plasmodium vivax*, but are even more bizarre in appearance and impossible of description. In these forms the chromatin is relatively large in amount and in the form of rods, patches, strands, or forked masses situated at the junction of the amœboid pseudopodia. Besides the "tenue" forms other forms are noted which are roughly round or oval in shape, stain deeply, and in which the chromatin consists of a rounded mass or circle situated within the vacuole. These forms may be young *gametes*, although Sinton does not mention this possibility. The infected erythrocyte is slightly larger than normal, and basophilic degeneration may be present.



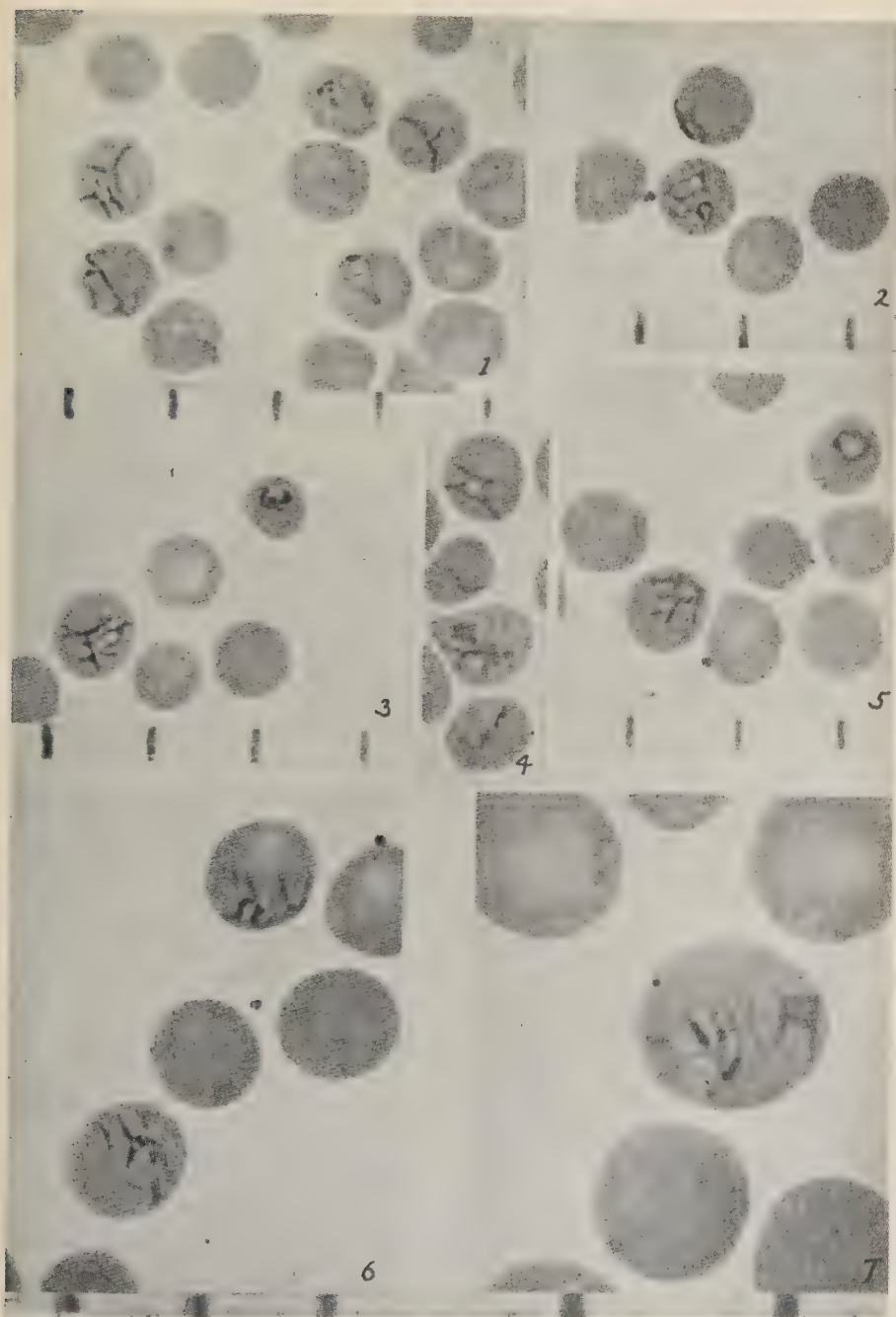


FIG. 81.—Photomicrographs of *Plasmodium tenue*. (After Sinton.) 1. Three "tenue" forms and one "tailed-oval" form. 2. An accolé and a "tenue" form. 3. An oval and a "tenue" form. 4. Three "tenue" forms. 5. An oval and a "tenue" form. 6. Two "tenue" forms. 7. A "tenue" form.

In from 36 to 40 hours the "tenu" forms have disappeared, and the plasmodia occur as oval or polyhedral bodies within the infected cell and occupying about one-half of the diameter. The cytoplasm stains a deep blue, and the chromatin occurs as a single round mass composed of granules or threads, or as a large compact dot, situated to one side of the vacuole. At this time a few yellowish-brown pigment grains, very minute in size, may be detected lying in the cytoplasm at that portion of the parasite furthest from the chromatin. At a little later stage of development the pigment is collected into a compact clump, and the chromatin shows evidences of division. The infected erythrocyte is smaller in size than normal, and basophilic degeneration is very evident, the entire surface of the cell being stippled with bluish staining granules.

Sinton observed but one segmenting, or sporulating, form, which contained 8 *merozoites* regularly arranged around a central clump of pigment, like the petals of a daisy, resembling in this respect the sporulating forms of *Plasmodium malariae*, the quartan plasmodium. The pigment was in the form of a loose mass of minute yellowish-brown granules. The sporulating form occupied about two-thirds of the diameter of the infected corpuscle and measured 5 microns in diameter.

The *merozoites* were oval in shape and measured about 1.5 microns in diameter. The centre of each *merozoite* contained a relatively large, purplish-red mass of chromatin dots surrounded by a narrow margin of deep-blue staining cytoplasm.

Sinton states that the *gametes* of *Plasmodium tenue* are crescentic in shape and resemble those of *Plasmodium falciparum*, but he does not give a detailed description of them.

**Habitat.**—So far as known, *Plasmodium tenue* lives only in the blood corpuscles of man.

**Species Occurring in Lower Animals.**—None of the species of plasmodia described as occurring in any of the lower animals resemble *Plasmodium tenue* in morphology, although Stephens believed that *Plasmodium canis* of the dog bore some resemblance to this species.

**Cultivation.**—*Plasmodium tenue* has not been cultivated.

**Life-history.**—In man the life-history of this species is like that of the other species of malaria plasmodia, *schizogony* being completed in 48 hours, at which time sporulating forms containing at least 8 *merozoites* are produced. Crescentic *gametes* are developed intended for the transmission of the infection to the mosquito. The life-cycle in the mosquito is unknown at present, but there is no reason to believe that it differs essentially from the life-cycle of the other malaria plasmodia in this insect.

**Geographical Distribution.**—The geographical distribution of *Plasmodium tenue* is unknown. The only region in which it has so far been found is in the Central Provinces, in India.

**Incidence of Infection.**—Unknown. It is evidently a rare form of the malaria plasmodia.

**Method of Transmission.**—Unknown, but reasoning from analogy, undoubtedly by mosquitoes belonging to the *Anophelinæ*. There is no experimental evidence bearing upon the method of transmission of this species of plasmodium.

**Experimental Infection of Lower Animals.**—No experiments have been recorded as to the possibility of infecting any of the lower animals with *Plasmodium tenue*.

**Relation to Disease.**—*Plasmodium tenue* is apparently the cause of a form of malarial fever presenting a tertian periodicity and rather mild symptoms, according to Sinton.

## Species II. PLASMODIUM OVALE, Stephens, 1922.

**History and Nomenclature.**—Stephens (1922) described a malaria plasmodium occurring in the blood of a patient who had contracted malaria in East Africa, the temperature curve being characterized by a tertian periodicity. The morphology of this plasmodium, as described by Stephens, is identical with that of *Plasmodium vivax minutum*, and in his description Stephens states that it resembled the latter species, but he regarded it as a new species, and proposed the name *Plasmodium ovale* for the organism.

There is little doubt that this plasmodium is identical with that described by myself, in 1900, and by Emin, in 1914, and named by him *Plasmodium vivax*, var. *minuta*, and that *Plasmodium ovale* is not entitled to specific rank. The name, therefore, becomes a synonym of *Plasmodium vivax minutum*.

**Morphology.**—All stages in *schizogony* occur in the peripheral blood of the patient. The youngest forms were indistinguishable from the "ring-forms" of other species of malaria plasmodia, but it was noted that they frequently occurred in oval-shaped erythrocytes. According to Stephens, the characteristic forms were the half-grown organisms which resembled the *schizonts* of *Plasmodium malariae*. These forms were spherical or oval in shape, and frequently were situated in oval erythrocytes. The "band" or "ribbon" forms, characteristic of *Plasmodium malariae*, were not observed, nor were irregular, bizarre-like forms, so characteristic of *Plasmodium vivax*, ever noted in the preparations. At this stage in the development of the plasmodium the cytoplasm of the infected erythrocytes frequently contained Schüffner's dots.

The segmenting, or sporulating, bodies almost filled the infected erythrocytes, and the *merozoites* varied in number from 6 to 12, the average being from 6 to 8. Schüffner's dots could be distinguished in the narrow rim of cytoplasm surrounding the sporulating forms. No *gametes* were

noted in the single case reported by Stephens, and a double infection of the erythrocyte was observed only once.

The illustrations which accompany Stephens' contribution, which are colored, are conclusive evidence that this plasmodium is identical with the one that I described in 1900, and, I believe, with Emin's plasmodium.

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## CHAPTER XVI

### THE PROPHYLAXIS AND DIAGNOSIS OF THE MALARIA PLASMODIA

**The Prophylaxis of the Malaria Plasmodia.**—In the prophylaxis of the malaria plasmodia the following methods have been found efficient: 1. The destruction of the mosquitoes transmitting the plasmodia; 2. The protection of man from the bites of the transmitting mosquitoes; 3. The destruction of the malaria plasmodia while in the blood of man. While theoretically any one of these methods should prevent malarial infection if it could be applied in an ideal manner, practically it is but rarely that one method alone is successful, and it is generally necessary to combine all of them in order to achieve the fullest success in the prophylaxis of the plasmodia.

1. **The Destruction of the Mosquitoes Transmitting the Plasmodia.**—The prophylaxis of malaria by the destruction of the mosquitoes transmitting the plasmodia *is the ideal method of prophylaxis* where conditions are such as to render it applicable, but in some regions, both in the temperate and tropical zones, natural conditions are such that it is either economically or physically impossible to destroy all anopheline mosquitoes, and the most that can be expected is to reduce the number of these insects. However, this method of prophylaxis should always be used to the fullest extent possible, and it will usually be found that excellent results will be obtained even in regions where conditions are apparently most unfavorable to this method of prophylaxis.

Before attempting the destruction of the mosquitoes concerned in the transmitting of the plasmodia a survey should be made of the region, and the breeding-places of these insects located. A map should be prepared and on it should be noted the breeding-places of all anopheline mosquitoes and data regarding the natural surroundings of these breeding-places and the possibility of getting rid of them. This mosquito survey is most important, but at the same time a careful study should be made of all local conditions and a decision reached as to the best way of preventing infections with the plasmodia. The blood of the apparently healthy children and adults should be examined, the results of which examination will demonstrate the amount of latent malaria present in the locality and the species of plasmodia that may be present. A careful palpation of the spleen of all children will also aid greatly in the determination of the amount of malaria present and the necessity for prophylaxis.

It is obvious that before one can intelligently apply methods of prophylaxis looking toward the destruction of malarial mosquitoes it is necessary that one have a good working knowledge of the life-history of

anopheline mosquitoes, and the following data will be found useful in this respect.

*The Differentiation of anopheline Mosquitoes.* All anopheline mosquitoes may be distinguished in both the larval, pupal, and adult stages of their life-cycle, and the ova of these insects are also distinct from those of other mosquitoes. The following practical points will prove of service in their differentiation.

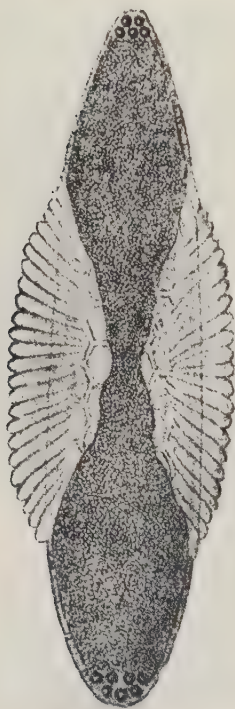


FIG. 82.—Egg of *Anopheles crucians*. Typical anopheline egg. (After Howard.)

1. *The Ova.* The ova of the *Anophelinæ* are never found in masses, as in the case of other mosquitoes, but are laid singly upon the surface of the water, the ends often being connected, thus forming geometrical patterns. They are further distinguished by the possession of lateral “floats,” which are never seen on the ova of other mosquitoes.

2. *The Larvæ.* The larvæ of the *Anophelinæ* are easily distinguished from those of other mosquitoes by the absence of the long respiratory tube or syphon. Because of this the anopheline larva is compelled to lie parallel with the surface of the water, while other mosquito larvæ hang head downward at an acute angle with the surface of the water. In addition, the small narrow head of the anopheline larva distinguishes it from other mosquito larvæ, in which the head is large and broad.

3. *The Pupæ.* While the pupal stage of development of the *Anophelinæ* can be distinguished from the pupæ of other mosquitoes, it is somewhat difficult to do so for one not trained in entomology, and for this reason the differential features will not be described, as the differentiation of the pupal stage is not of practical importance.

4. *The Imago or Adult.* The following serve to distinguish adult anopheline mosquitoes from other adult mosquitoes:

a. *The relative length of the palpi and the proboscis.* In all *Anophelinæ* the palpi of the female are as long as the proboscis, while in other mosquitoes the palpi are much shorter than the proboscis. By attention to this simple detail any mosquito may at once be placed, so far as its relation to the *Anophelinæ* is concerned.

b. *The angle of the proboscis with the body.* The proboscis in anoph-

eline mosquitoes never forms an angle with the rest of the body, but continues in a straight line with it.

c. *The resting position.* When resting upon a surface most of the *Anophelinæ* form a distinct angle with the surface, while other mosquitoes appear humpbacked, owing to the fact that the abdomen is nearer the surface upon which the insect rests than is the thorax. Usually anopheline mosquitoes rest upon the first two pairs of legs, the last pair floating in the air or held out straight behind, while the abdomen, thorax, and proboscis form a straight line. The angle formed by the body of the mosquito



FIG. 83.—*Anopheles* and *Culex* larvæ in natural position in water. (After Howard.)

with the resting surface varies in different species, but is sometimes nearly a right angle. The only exceptions to this rule among the common anopheline mosquitoes are *A. culicifacies*, which appears humpbacked when resting, and *A. superpictus* and *A. hispaniola*, which assume a perpendicular position in reference to the resting surface.

d. *The spotted wings.* Most of the *Anophelinæ* have spotted wings, and while some other mosquitoes present spots upon these structures it is a good practical rule to always regard with suspicion any mosquito having spotted wings, as the chances are altogether in favor of its being an anopheline.

Attention to the points enumerated above should enable one to differentiate anopheline mosquitoes from other mosquitoes, but the differentiation of the various species of anophelines should be made by a trained entomologist.

The malaria mosquitoes may be destroyed at any stage in their development, but most of the prophylactic methods depend for success

upon the destruction of the insect during the larval and adult stage, the larvæ being destroyed by the abolition of the collections of water in which they have developed, or by larvicides, and the adult insects by various chemical agents or other measures. The most important measures consist of the abolition of the breeding-places of the mosquitoes by leveling, drainage, and clearing, and if these measures can be carried out in an efficient manner they are the most valuable that we possess.

If possible, all large breeding-places of mosquitoes should be *drained*, if they cannot be filled in or otherwise abolished. If filling in is feasible, this is the better method, but the relative cost of the two methods should be considered for each locality, and the choice made as to which will be the cheapest and most efficient. *Drainage* may be by open ditches, cement-lined drains, blind drains, or subsoil drainage with tiles. The results obtained upon the Canal Zone with drainage speak eloquently of the value of this method of malaria prophylaxis, and during the World War our camps in the malarial regions of the southern United States were rendered practically free from malaria largely through the destruction of the breeding-places of mosquitoes in the adjacent localities by drainage and filling in.

The removal of *shelter for the mosquito*, or clearing, is a most important prophylactic method, and should always be used in conjunction with drainage or filling in, and as a separate method. The removal of brush along streams and of vegetation in the streams, or of any shelter for mosquitoes, such as is furnished by jungle, long grass around residences, and vines and bushes, will be followed, in most instances, by a very sensible reduction in the number of mosquitoes in the locality. Shrubbery and vines about habitations in malarial regions, unless the mosquitoes can be eradicated by other measures, furnish shelter for the insects, and their removal will result in a marked reduction in the amount of malaria through reducing the number of mosquitoes. On the Canal Zone it was found that clearing for a distance of 200 yards around the houses was followed by excellent results, this distance being adopted under the erroneous belief that anopheline mosquitoes did not fly for any great distance to obtain a feeding of blood. Although good results were obtained by the method, it was not because the insects would not fly for a greater distance than 200 yards, for it has been definitely proven that anopheline mosquitoes will fly for a distance of over 2 miles, if necessary, in order to obtain blood. In 1906, I stated that at Camp Stotsenberg, in the Philippine Islands, the anopheline mosquitoes flew over two miles in order to secure a feeding of blood, as breeding-places were at least that distance from the post. This statement was received with incredulity by entomologists, but it has since been abundantly confirmed by observations upon the Canal Zone, and in our camps during the World War. The experiments of Darling and others in the Canal Zone (1919-1920) and of



Bascom (1917-1918) at Ebert Field, Ark., have proven that anopheline mosquitoes fly from 2 to 2.5 miles frequently in search of food, and Bascom found that the usual flight was from one-half to one and a quarter miles. Wenyon (1921) found the same to be true of the flight of anophe-

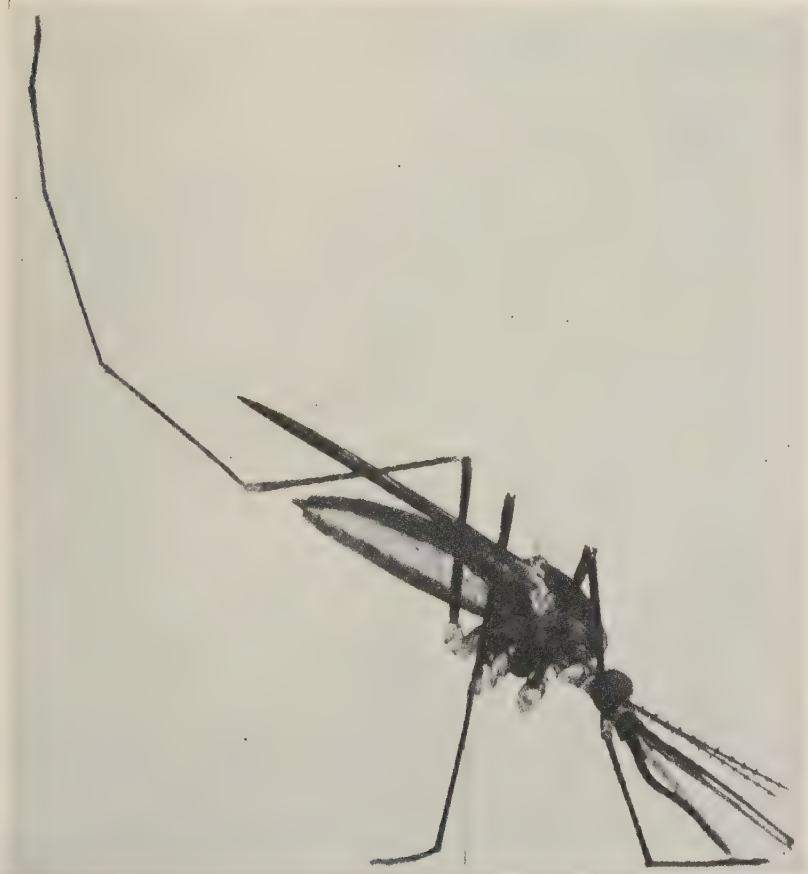


FIG. 84.—Female anopheles in characteristic biting posture. (Photograph of model in the American Museum of Natural History.)

line mosquitoes in Macedonia, and states: "The range of flight, especially when it can be done in stages, is not half a mile, but often two or three miles or even more. The absence of a population near a breeding-place encourages the mosquitoes to travel long distances to find a host upon which they can feed. The result is, that the camps, though surrounded by a cleared area of half a mile, even if this area were properly maintained, became the centre of attraction for mosquitoes breeding all over the country."

From these observations it is evident that clearing alone will not pre-

vent the access of mosquitoes, but it undoubtedly greatly reduces the number by making it impossible for the insects to obtain shelter.

*The abolition of small breeding-places about habitations* is of great importance in prophylaxis, as some of the anopheline mosquitoes transmitting malaria will breed in small collections of water in and about human habitations. This fact renders the sanitation of such habitations most important, and such breeding-places may be easily discovered and destroyed. Among the most common of the smaller breeding-places may be mentioned fire buckets that are not emptied at least once a week, stopped drains or roof gutters, unused hoppers in toilets, unscreened water tanks, tubs, tin cans containing a little water, water traps that are not flushed out frequently, vessels used for the storage of water, and the cans or dishes of water that are often used in the tropics to immerse table legs in to prevent the access of ants. All of these are favorite breeding-places of certain species of anopheline mosquitoes, and are frequently overlooked by those having little knowledge of the habits of these mosquitoes.

The native quarters in the vicinity of European habitations are especially apt to be sources of mosquitoes, and their sanitation is necessary. An inspection of native premises will generally reveal larvæ in the water that has collected in broken bottles, old culinary utensils, tin cans, tin-lined boxes, water barrels, and chicken troughs. In addition, puddles of water are usually found teeming with larvæ in the immediate vicinity of the native houses, and even the banana trees and bamboo fences that surround so many native houses will be found breeding mosquitoes, the insects developing in the small cavity filled with water at the base of the banana leaves, and in the cavities left by sawing off the bamboo near a joint, or within the bamboo where mosquitoes have gained entrance through holes made by boring insects, and where a small amount of water has accumulated.

The discovery of these small breeding-places of the *Anophelinæ* and their destruction or proper treatment forms one of the most important duties of sanitary officers in malarial localities. All receptacles in which mosquitoes can breed and which are not needed in domestic operations should be removed or destroyed, and those in which it is necessary to keep water should be either frequently emptied, screened, or oiled. Barrels and tubs for the collection of rain-water, as well as cisterns, should be covered with a perfectly fitted screen cover, in which the netting, made of wire, should contain at least 18 meshes to the linear inch. Unused hoppers of toilets, and water traps, should be flushed thoroughly at least once a week, and drains should be frequently inspected and flushed. One of the most common sources of mosquitoes around habitations are roof gutters which have become clogged with material and allowed the accumulation of water. I have frequently found such gutters alive with larvæ, and the inspection and proper sanitation of roof gutters

is most important in prophylaxis. Fire buckets should be emptied once a week, and if this is done there is no danger of them acting as breeding-

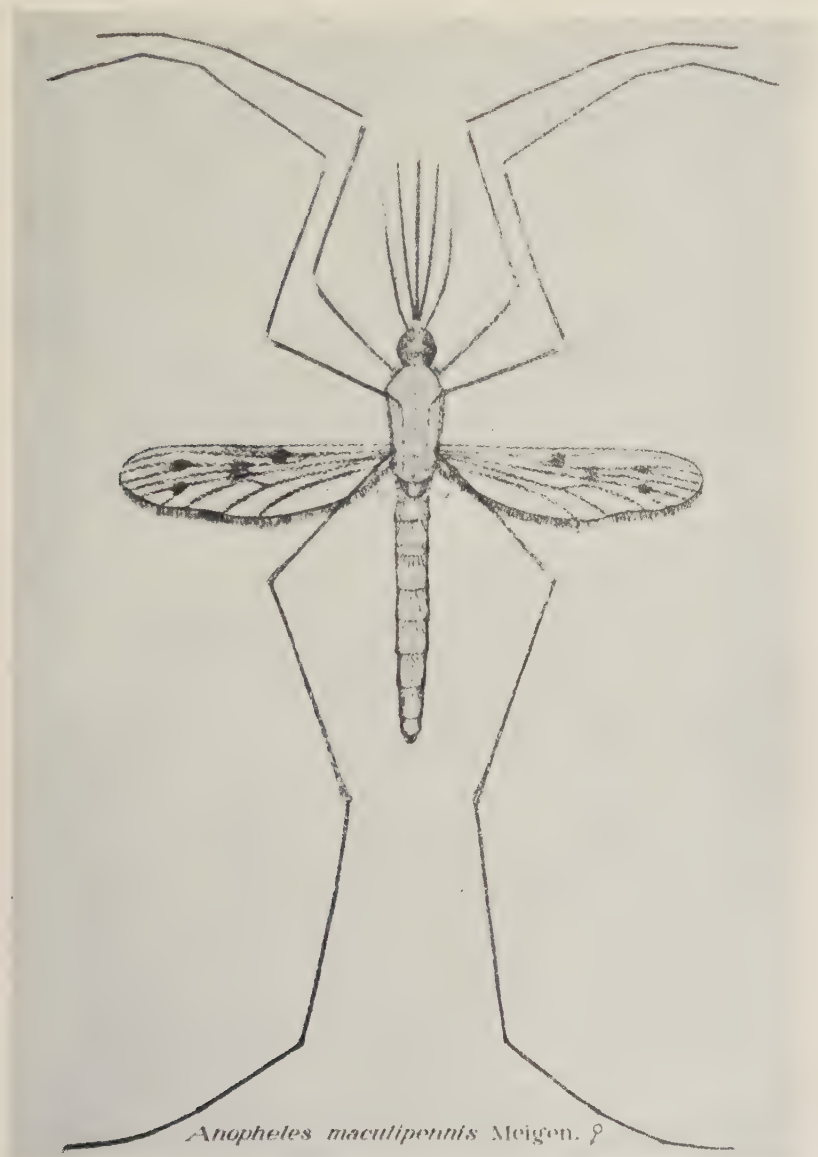


FIG. 85.—Typical anopheline mosquito. Note long palpi and spotted wings. (After Ludlow.)

places of mosquitoes. It is not necessary to screen fire buckets, or to oil the water contained within them, if they are properly emptied. The buckets should not be emptied into a sewer, but upon the ground in

direct sunlight, where the water will evaporate quickly and the larvæ be killed by the sun.

Water troughs used for domestic animals should be flushed frequently, and in no case should the water be allowed to remain in the trough if it is

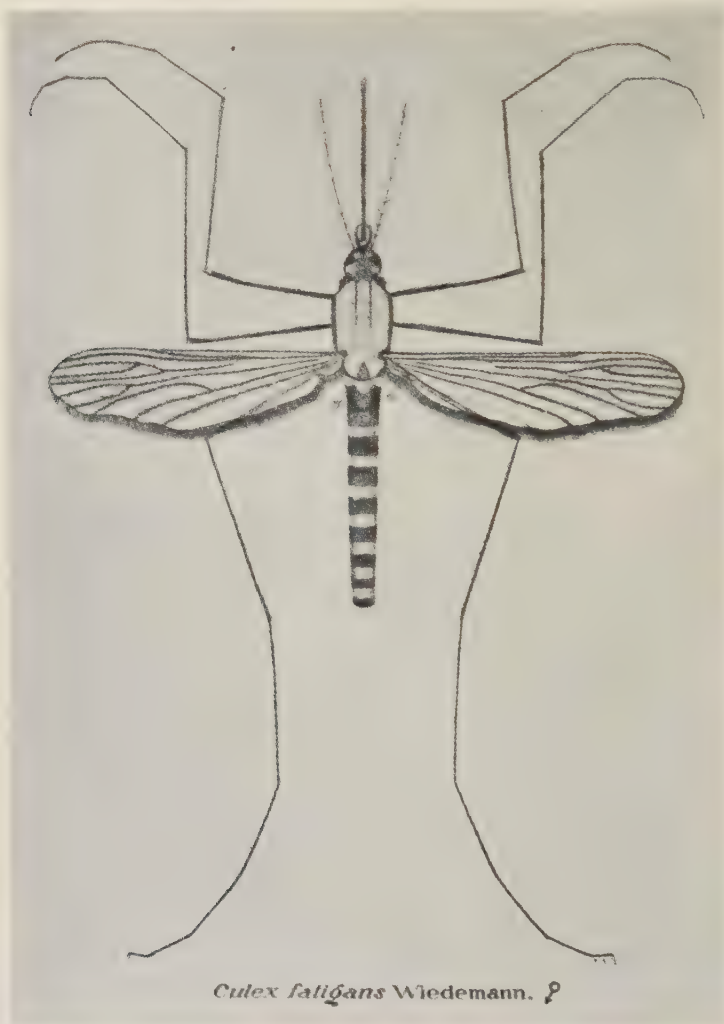


FIG. 86.—Typical culicine mosquito. Note short palpi and unspotted wings. (After Ludlow.)

not in use. The tanks in water-closets, the sewer traps, and often cesspools, if present, should be frequently inspected and properly treated, the tanks and sewer traps by flushing or oiling, and the cesspools by oiling at intervals of two weeks.

Where the breeding-places of anophelines cannot be drained, filled in,



or otherwise destroyed, the *destruction of the larvæ with larvicides* is a prophylactic measure of considerable value. For this purpose the substances most used are petroleum, or crude oil, and the Canal Zone larvicide, devised by Darling.

The use of kerosene as a larvicide was first recommended by Howard (1893), and it has been used very extensively in malaria prophylaxis, but has been largely replaced by the use of crude oil, or green oil, as it is sometimes called. The success of this method depends upon coating the surface of the water in the breeding-place with a layer of oil which prevents the larvæ from breathing at the surface, and thus results in their death. An oil should be selected that will spread with a thin, even film, and experience has shown that the grade of petroleum known as light fuel oil has been found satisfactory, and a thicker oil, composed of 4 parts of oil of 18° gravity and 1 part of oil of 34° gravity, is also excellent.

The application of the oil, in the case of large bodies of water, is best made with a hand pump having a straight nozzle. The operator is rowed from side to side of the collection of water and the oil discharged at such intervals that when it rises and spreads the entire surface of the water is covered. In smaller collections of water an ordinary watering can equipped with a spray nozzle may be used to distribute the oil, or the latter may simply be poured from a cup or dipper. The greatest care should be taken to see that there are no portions of the surface left uncovered after the oil has spread, and all oiled collections of water should be inspected at least every third or fourth day, as the layer of oil is easily displaced by currents in the water, by winds, or by the movements of aquatic animals. Whenever unoiled areas appear upon the surface the oiling of that area should be repeated. In the case of more or less stagnant streams or ditches the oil is best applied with a pump, and for this purpose the ordinary knapsack pump or bucket pump is satisfactory.

The time for renewing the oil varies under different natural conditions. While, in rare instances, one application of oil may be sufficient for as long a period as four weeks, in the vast majority of cases the application will have to be renewed much within that time. Where the temperature is high, and the surface of the water is exposed to the sun, the oil evaporates rather rapidly and has to be renewed at much shorter intervals than where the temperature is low and the surface of the water well shaded. In some regions oiling is rendered impossible by reason of constant high winds or the growth of tropical vegetation in the collections of water.

The amount of oil to be used will vary with local conditions and the character of the oil available. Usually half an ounce of oil per square yard of surface will be found sufficient, and it is good practice to renew the oil regularly once a week.

Where it is impossible to use oil, or it is too expensive, the Canal Zone

larvicide, composed of a mixture of crude carbolic acid, rosin, and caustic soda, devised by Darling (1912), has been found very efficient, the mixture killing mosquito larvæ in five minutes. It is used by spraying an emulsion composed of 1 part of the larvicide to 5 parts of water over the surface of the water and along the edges of ponds, streams, or other collections of water that serve as breeding-places. This larvicide possesses the advantage that after a few hours, in running streams, the water will be again fit for domestic use.

Where it is necessary to keep a slowly running small stream constantly oiled, the drip barrel is of great service, the drip being carefully regulated to the supply of oil sufficient to accomplish the purpose.

Other larvicides that have been employed, with more or less success, are permanganate of potassium, sulphate of copper, corn oil, and Phinotas oil, but none are so efficient as petroleum or the Canal Zone larvicide. Phinotas oil kills fish, so that it should not be used if it is desired to preserve the latter.

Although it is possible to secure excellent results by the use of larvicides, the adoption of this method of prophylaxis is always an admission that we are unable to control mosquito breeding by the much better method of abolishing the breeding-places of the insects, and it should never be adopted in preference to the latter, but always used as a complement. Under the best of conditions the method is expensive, uncertain, and requires constant vigilance to render it effective, and it finds its greatest field of usefulness in those instances where breeding-places cannot be abolished owing to expense involved, or where open ditches or streams cannot otherwise be prevented from becoming breeding-places.

*The destruction of mosquito larvæ by fish* is a prophylactic measure of considerable value. Certain species of fish are voracious devourers of mosquito larvæ, and stocking pools, lakes, and slowly running streams with these fish has proven efficient, in many instances, in ridding the water of larvæ. It is said, upon good authority, that the freedom of Barbados from malaria is due to the prevalence of certain species of fish in the waters of that island, which feed upon the mosquito larvæ to such an extent that the insects are never numerous enough to cause infection. The fish that have been found most useful are the roach, carp, top minnows, and goldfish, the roach and top minnow especially being very active in devouring the larvæ. This method of prophylaxis is useful in the case of small collections of water around houses, as fountains, ornamental pools, or small ponds, and should be adopted in conjunction with the other methods of malaria prophylaxis, but never depended upon alone.

*The destruction of the adult mosquito* is a valuable method of prophylaxis of the malaria plasmodia, and may be accomplished by various

chemicals, generally used as fumigants, and by catching either in traps or by hand.

A large number of chemicals have been recommended for the destruction of adult mosquitoes, the most important of which will be briefly discussed.

*Sulphur dioxide.* When it is desired to kill mosquitoes in barracks, quarters, on ships and transports, or in habitations in malarial regions, sulphur dioxide is the most valuable chemical agent that is available for the purpose. The success attending its use in Cuba, Panama, Rio Janeiro, and Santiago in the fumigation of houses infested with the yellow fever mosquito, is familiar to all sanitarians, and the same methods that were used against the yellow fever mosquito are equally valuable in the destruction of the mosquitoes transmitting the malarial fevers.

In employing this agent it should be remembered that it injures fabrics and most metallic substances, and these should be removed before fumigation with it is begun. The amount of sulphur necessary to destroy mosquitoes depends upon the size of the room or building to be fumigated, and is ascertained by dividing the number of cubic feet in the room or building by 500 and reading the result in pounds. Thus, a room 40 feet long, 20 feet wide, and 12 feet high would contain 9,600 cubic feet, and this divided by 500 would give 19.2, or 19.2 pounds of sulphur for a room of that size.

Another chemical which has proven useful in killing mosquitoes is *cresol*. This may be vaporized in a tin over a flame, and 4 ounces (120 c.c.) should be used for each 1,000 cubic feet of room space.

*Pyrethrum powder* has been used for generations in the destruction of the adult mosquito, and is very efficient when properly used. This powder is made from the dried flower heads of plants belonging to the genus *Chrysanthemum*, growing in Transcaucasia. The powder is more commonly known as Persian or Dalmatian insect powder, and depends for its efficacy on the presence of certain oleoresins in the dried flowers, so that to be efficient it should be fairly fresh. The powder is burned in the room to be fumigated, being placed upon a dish or tin, and a match touched to it, when it will burn slowly, giving off a large volume of smoke having a peculiar odor; or the powder may be procured in the form of cones or pastilles, and these burned. The smoke is not harmful or very unpleasant to most people, and if the powder be burned in a closed room it will stupefy all mosquitoes present, and they may be swept from the floor and destroyed.

Among other chemical agents that have been used for the destruction of the adult mosquito may be mentioned camphor, phenol, mercuric chloride, powdered stramonium, and formaldehyde gas, but none of these are as efficient as sulphur dioxide or pyrethrum powder. This method of prophylaxis can only be regarded as a makeshift, and should never be

employed to the exclusion of the destruction of the insects in the larval stage of development, so far as malarial infection is concerned.

*The destruction of mosquitoes by catching the adults* is deserving of consideration as a prophylactic measure, as it is a method that is easily applied and requires no initial outlay beyond the cost of traps and tubes for catching the mosquitoes. The insects may be caught by hand in the chloroform or cyanide tube, or by traps which are fixed to habitations. The method was given a very thorough trial by Orenstein (1913), in the Canal Zone, and his reports indicate that excellent results were obtained.

The insects are caught by inverting a test tube, or small glass cylinder, having cotton saturated with chloroform or the cyanide mixture in the bottom, over the insect and gently stirring the tube, when the mosquito will fly to the further end and be stupefied. In this way a single individual can catch scores of these insects within a short time, and thus greatly lessen the chances of infection with the plasmodia. The mosquitoes should be searched for in the dark corners of rooms, in closets, along base-boards, beneath sinks and toilet fixtures, behind pictures, in corners behind clothing, and beneath cots and beds, while in tents the most frequent lurking-places of these insects are along the ridge-poles and beneath cots and mosquito nets. The mosquito-catcher should also be equipped with a "fly-swatter," with which he will be able to "swat" many of the mosquitoes that he fails to catch in the tube. The "fly-swatter" is especially useful for killing mosquitoes so frequently observed on the outside or inside of screening on porches, doors, and windows. At Camp Stotsenberg, in the Philippine Islands, I have frequently seen hundreds of anopheline mosquitoes resting upon the outside of the window screens endeavoring to get into the buildings, and it was possible to literally kill thousands of them in this situation almost every evening during the wet season by means of the "fly-swatter," if a round of the screened buildings of the post was made for this purpose.

*Mosquito traps* have also been used extensively in some localities, especially in the Canal Zone. These traps are so placed as to catch the mosquitoes when entering or leaving a building, and in the Canal Zone it was found that if the traps were placed on the lee of buildings more anophelines were caught than culicines, the opposite being true if the traps were placed upon the windward side of the buildings. A trap which proved very successful, and which was designed by Sanitary Inspector Bath, consisted of a half-cylinder constructed of wire netting, 18 meshes to the linear inch, and having two ridges, the apices of which were perforated by longitudinal slits about  $\frac{1}{4}$  inch wide and 3 inches long. The mosquitoes entered the trap through these slits, and were unable, for some reason, to find their way out again.

Orenstein concluded, as the result of his experience in the Canal Zone,



that catching the adult mosquito was a measure of very great prophylactic value against malaria, and a method that should be employed wherever mosquitoes are prevalent in habitations. It is obvious that this method would be of great service in military camps in malarial regions, and its neglect would be censurable. All that would be necessary to put it in operation under such conditions would be to detail a certain number of men for the purpose, under an intelligent non-commissioned officer, the camp being districted for the purpose. No great amount of training is necessary, as mosquitoes are easily recognized and the method of handling the killing tube and "fly-swatter" is quickly acquired, even by the most unintelligent laborer. In the military service this method should prove of much value in the field and in barracks and quarters in posts situated in malarial localities.

As regards the relative value of the various methods of prophylaxis based upon the destruction of the mosquitoes transmitting the malaria plasmodia, it may be stated that the abolition of the breeding-places of the insects is of greatest importance, followed in turn by the destruction of the larvæ and of the adult mosquito. Of the methods used for the destruction of the larvæ the use of oil or some equally efficient larvicide is of first importance, while of the methods used for the destruction of the adult insect fumigation with sulphur dioxide is of greatest value. It will be found in practice that usually more than one method will have to be employed to achieve success, and in some regions all methods will be found necessary, but the greatest efforts should be made along the line of the abolition of the breeding-places of the mosquitoes.

**2. The Protection of Man from the Bites of Mosquitoes Transmitting the Plasmodia.**—Where it is impossible to destroy all of the breeding-places of mosquitoes transmitting the malaria plasmodia, or to kill the adult insects, the protection of man from the bites of these insects becomes a most important prophylactic method. While, as has been stated, the abolition of breeding-places is the ideal method of malaria prophylaxis, and other methods should never be substituted for this where it is possible to employ it, the fact remains that in most localities it is necessary to combine all the available methods of prophylaxis, and of these the protection of man from mosquito bites is one of the most valuable.

This mechanical prophylaxis, as it is sometimes called, is secured by the proper screening of habitations, the use of mosquito nets for beds and in tentage, the wearing of head nets and gloves, and the use of various substances smeared upon the skin which have a repellant odor to mosquitoes.

*The screening of human habitations* should never be neglected in regions where malaria-transmitting mosquitoes are present and the breeding-places cannot be totally abolished. The method is rather expensive, but this should not militate against its use, as the reduction of malaria brought

about by its use will more than compensate for the expense involved, and in the end will demonstrate that screening is actually an economical measure. If the wire netting used in screening is of good quality it will last, if properly cared for, for several years, and care should be used in selecting material for this purpose to see that it is of first-class quality. The material of which the netting is composed should be composed of copper, zinc, and iron, the copper content being higher than that of brass. In the tropics this is of special importance, as the heat and moisture rapidly corrode wire netting having a low percentage of copper. The deterioration of wire screening in the tropics has been found by Darling to be due to the presence in the netting of iron, plus the influence of a hot and moist climate. Therefore, brass screening is not suitable by reason of the amount of iron alloy present, and copper screening should always be selected. If copper screening cannot be secured ordinary iron-wire screening may be used, but it should be protected by at least two coats of good paint, and frequently repainted, but, even so, iron screening will be found more expensive in the end than copper screening.

The *size of the mesh* in the wire screening used to protect habitations is a matter of prime importance. It should be close enough to keep out all anopheline mosquitoes and, for comfort, and in regions where the yellow fever mosquito is present, the mesh should be small enough to keep out the yellow fever mosquito, *Aedes aegypti* (*Stegomyia fasciata*), and the common culicines. However, it should be remembered that nothing is gained by using a netting in which the meshes are smaller than necessary, and much is lost, for the smaller the mesh the more air is excluded from the building, a matter of importance, especially in the tropics.

As a result of my own observations in the Philippine Islands I (1909) concluded that wire netting containing 16 meshes to the linear inch was sufficient to exclude all species of anopheline mosquitoes encountered in these islands, and that this size netting should be used in malaria prophylaxis. My observations have been confirmed, so far as anopheline mosquitoes are concerned, by Guiteras (1912), Darling (1912), and the United States Army Board for the Study of Tropical Diseases in the Philippines (1912). This size netting (No. 16) is not efficient in excluding the yellow fever mosquito, as the majority of investigators have shown, and a netting containing 18 meshes to the linear inch should be used, as shown by Darling (1912) and the Board for the Study of Tropical Diseases (1912).

It is very probable that mosquitoes vary in size, even when full-grown, for it has been shown that delayed development during the larval stage results in dwarfed mosquitoes, and this doubtless accounts for some of the diverse results obtained by different investigators as to the efficiency of various sized wire netting. Guiteras (1912) reported, as the result of

careful experiments, that No. 16 netting was small enough to exclude the yellow fever mosquito, but Darling (1912), as the result of his experiments, stated that while No. 16 netting was practically safe, it was not absolutely so as regards the exclusion of the yellow fever mosquito. He found that none of the anophelines in the Canal Zone could pass the No. 16 netting (16-mesh), but that some specimens of *Ædes ægypti* (*Stegomyia fasciata*), as well as several species of *Culex*, could do so. The Army Board for the Study of Tropical Diseases (1912) found that 16-mesh netting excluded all anopheline mosquitoes, but that it did not exclude the yellow fever mosquito, and recommended that for the protection of habitations an 18-mesh netting be employed. As it is essential to protect man not only against malaria but also against yellow fever and dengue, it would appear that the use of the 18-mesh netting should be preferred, though a netting containing 17 meshes to the linear inch will probably be found perfectly safe in protecting man from all of the diseases mentioned.

*The method of using wire screens* is of great importance. Any one who has inspected screened buildings in malarial regions is aware of the fact that much of the screening is so constructed as to defeat, in a measure, the purpose intended, *i.e.*, the exclusion of mosquitoes. The use of adjustable or fold screens; the placing of the screen within the window, necessitating its being raised every time that the window is opened; and the use of the single screened door, are all instances, commonly observed, of defective screening.

The arrangement of the screens will vary, of course, with the architecture of the building to be screened, but all window screens should be placed outside window-sashes, and fitted as closely as possible to the sash, as otherwise mosquitoes may easily get in between the screen and the sash, especially when the window is partially open. In fact, to insure perfect protection the entire window space should be covered externally by a single screen which is attached so that it cannot be moved without unscrewing the screws which should hold it in place. In this way either the upper or lower sash may be lowered or raised without any danger of admitting mosquitoes. All entrances to buildings should be protected by a screened vestibule containing two screened doors, instead of the single screened door so frequently observed, and all screened doors should open outward. Extreme care should be taken that all ventilators be screened, especially the ventilating space beneath the roof so general in buildings in the tropics. Screening should be inspected frequently and repaired promptly when necessary.

Where wire screening is impossible, because of the expense involved, screening of doors and windows with cheese-cloth screens may be employed. Such screening, while not very durable, is satisfactory if carefully watched for the appearance of holes or tears and promptly repaired.



When it is impossible to thoroughly screen habitations, and when troops are in the field or in temporary camps, or one is camping, the mosquito net, or mosquito bar, as it is commonly called, furnishes a valuable means of protection from the bites of these insects. In habitations mosquito nets are used over the bed, and during the day the net should be taken down and folded up on the bed, but it may be tucked in around the bed or carefully rolled upon the portion suspended between the uprights. At night the mosquito net should be carefully tucked in beneath the mattress, when in use, for if it be allowed to hang loosely about the bed mosquitoes easily gain entrance by crawling or flying between the net and the mattress. Portable mosquito nets, for use in camping, are now commonly available, and for use by troops in the field the net devised by Lieutenant Colonel Vedder, U. S. Army, which can be used with the shelter tent, is admirable.

*The use of head nets and gloves* is absolutely necessary in many regions if one is camping, fishing, or hunting, and the same is true when troops are in the field in such localities. Head nets can now be purchased without trouble, and an excellent head net is a very valuable companion where mosquitoes are prevalent, if only for the comfort afforded. Gloves should be worn if mosquitoes are prevalent when one is exposed to their bites in the open.

*The use of odorous substances upon the skin* is a common practice in regions where mosquitoes are numerous. Various substances have been recommended, all of them based upon the fact that their odor is obnoxious to mosquitoes. Among the best may be mentioned the oils of citronella, eucalyptus, pennyroyal, anise, and camphor, vaseline, and kerosene. For use as a temporary measure a mixture of oil of citronella and liquid vaseline is very efficient in preventing mosquito bites, and is not unpleasant to use. One part of oil of citronella should be mixed with six parts of liquid vaseline, and the mixture applied frequently to the exposed parts of the skin. If liquid vaseline cannot be obtained, ordinary vaseline can be used, a teaspoonful of the oil of citronella being mixed with two ounces of vaseline.

It is obvious that the use of these various odorous substances is a poor substitute for screening, and as they are only temporary in protective effect and depend entirely upon the will of the individual for what success they may have, they are not of great value in the prophylaxis of the plasmodia. The use of such substances should be rendered unnecessary by the adoption of other, and more efficient, prophylactic methods.

*The screening of patients with malaria* is a prophylactic method of prime importance. As long as plasmodia can be demonstrated in the peripheral blood of patients, the greatest care should be taken to prevent mosquitoes from gaining access to such patients, and this can only be done by placing them in screened rooms or wards, or beneath mosquito nets. It



is obvious that if patients with gametes of the plasmodia be exposed to the bites of anopheline mosquitoes, the mosquitoes will become infected, and, in turn, will infect other individuals. If this does not occur because of the absence of gametes, the mosquito may reinfect the patient, provided it happens to be infective, so that screening is necessary not only to prevent the infection of others, but also to protect the patient from reinfection. These facts would appear to be too obvious for any one to disregard, but I have repeatedly observed malarial patients in wards unprotected by screening and without mosquito nets, although malaria was endemic in the locality and anopheline mosquitoes were numerous.

If the ward or room in which the malarial patient is confined be screened properly it is unnecessary to further screen the patient, but if not, the patient should be kept beneath a mosquito net until the plasmodia have disappeared from his peripheral blood, or until the gametes have been reduced to a non-infectious minimum. This procedure necessitates a microscopical examination of the patient's blood at daily intervals, but it is only in this way that we can render the prophylaxis of the infection effective, so far as the patient is concerned. The screening of every individual suffering from malarial fever, and in whom the plasmodia are present in the peripheral blood is an absolutely necessary prophylactic measure if one expects success in the limitation of the spread of the malaria plasmodia.

3. **The Destruction of the Malaria Plasmodia.**—*Quinine Prophylaxis.* It is obvious that if the malaria plasmodia in the blood of infected individuals can be destroyed, or their development prevented, we will succeed in preventing malarial infection, as anopheline mosquitoes could not become infected. This is the basis of quinine prophylaxis, which consists in the administration of this drug to all individuals harboring the plasmodia, and to all those exposed to the bites of mosquitoes capable of transmitting the plasmodia to man.

Perhaps no method in the prophylaxis of malaria has caused more controversy than the use of quinine as a prophylactic. Enthusiasts in its favor have claimed that the proper use of this drug alone is all that is needed to eradicate the plasmodia from any locality, while its most violent enemies have claimed that it is not only useless but harmful. As a matter of fact, the truth lies between these extreme opinions, and there can be no doubt that, when properly applied, quinine prophylaxis is a valuable method, but that, as usually applied, it is only a poor substitute for the methods based upon mosquito destruction. It is also true, that where mosquitoes are very numerous and there are many malarial infections, quinine prophylaxis often fails to prevent many cases of infection, because, under such conditions, the individual is continually being bitten by infected mosquitoes, and the doses of quinine used in prophylaxis are insufficient to kill all of

the plasmodia injected by the mosquitoes. In other words, where the dose of plasmodia is excessive, quinine prophylaxis frequently fails to prevent infection.

From the observations of many investigators, as well as from clinical experience, we know that quinine is capable of destroying the malaria plasmodia while they are in the blood of man, and that their destruction leads to the recovery of the patient. To deny that the drug, when properly administered, will kill the plasmodia before the appearance of symptoms, if present in the blood, is illogical and absurd, for if this drug will kill the plasmodia in the sick individual, and cure the infection, it will certainly destroy them in those individuals in whom they are not yet numerous enough to produce symptoms. However, experience has shown that a larger dose must be used for this purpose than was formerly believed to be necessary, and that much depends upon the dosage of the plasmodia and the condition of resistance of the individual. If many plasmodia are injected by numerous infected mosquitoes the dose of the drug used in prophylaxis is often insufficient to prevent infection and the development of symptoms.

While there are ardent advocates of this method who claim that with quinine it is possible to entirely eradicate the malaria plasmodia from a locality, it has never been proven practically that this is possible. No doubt a great reduction may be obtained in the number of malarial infections by this method of prophylaxis alone, but that it can, under natural conditions, entirely rid a locality of malaria, is doubtful. Quinine prophylaxis, or the destruction of the malaria plasmodia, is a very valuable prophylactic method when properly applied, but it should always be combined with methods for the destruction of mosquitoes and never depended upon alone, except under certain circumstances, as in camping in, or exploring, regions where malaria is present, or in the case of troops marching and camping in such localities.

*True* quinine prophylaxis in malaria consists in the administration of quinine to individuals who have never suffered from malaria in doses sufficient to prevent their becoming infected with the plasmodia. This is a very different matter from giving the drug to individuals who have been infected, in order to prevent relapses, which is sometimes called quinine prophylaxis. To prevent relapses requires larger doses and a more prolonged administration of the drug than does the prevention of initial infections, and a clear distinction should be made between the prophylaxis of initial infection, or true quinine prophylaxis, and the prophylaxis of relapses, which is often confused with true prophylaxis.

Among the arguments that have been urged against quinine prophylaxis may be mentioned the production of hæmoglobinuria; a harmful effect upon the individual taking the drug; the difficulty of properly apply-

ing the method; and the possible danger of producing resistant strains of the plasmodia by the exhibition of small doses of quinine over a long period of time. None of these objections are valid. Hæmoglobinuria is rarely produced by the drug in persons having an idiosyncrasy to it, but not sufficiently often to forbid its use as a prophylactic. There is no scientific proof that the exhibition of moderate doses of quinine produces quinine-fast strains of the plasmodia, while evidence that taking the drug for long periods of time results in harm to the consumer is likewise lacking. The objection that the method is difficult of application is a very poor excuse for its neglect. Every health officer should be thoroughly familiar with the various methods of applying this form of prophylaxis, which, to be successful, must include a knowledge of the best form in which to give the drug and the best time of administration. Quinine prophylaxis also includes, in its broadest sense, the proper treatment of initial infections, of latent infections, and of gamete carriers, so that an intimate knowledge is required of each of these conditions.

*The form of quinine to be used in prophylaxis* has been a subject of considerable controversy, but I believe, from experience, that the only salts of quinine deserving consideration in this respect are the sulphate, the tannate, and quinine alkaloid. Of these the sulphate and the tannate have proven most generally useful, and it may be stated that the sulphate, while not the most ideal form of quinine for the purpose, is the most generally used, and with it results are adequate when its administration is properly controlled. Quinine alkaloid possesses the advantage of being almost tasteless, low in cost, and, being pure quinine, representing the drug in the smallest bulk and weight, and it has been found efficient in the prophylaxis of the plasmodia, but, for practical purposes, the sulphate is the form in which quinine is best used in prophylaxis. The tannate of quinine is especially useful for administration to children, as it is almost tasteless, but it is expensive, especially when used in prophylaxis.

*The method of administration of quinine in prophylaxis* is of much importance, and the *dosage* is equally important. The methods that follow are intended to protect the individual from infection by the plasmodia, and not to prevent relapses or treat "carriers" of gametes.

All methods of quinine prophylaxis may be divided into two classes, those in which the drug is given daily and those in which it is administered at longer intervals. The latter methods are all aimed at doing away with the irksome daily administration, but all have the fault that one must remember the days upon which quinine is to be given, with the result that many doses are missed, while there is good evidence that none of them are as efficient as the daily administration of the drug.

Celli recommended the administration of 40 centigrams (6 grains) of quinine daily, 20 centigrams in the morning and 20 centigrams in the



evening. Half of this dose was administered to children. Sergeant recommended a daily dose of 20 centigrams (3 grains), while others have recommended a daily dose of even less than 20 centigrams. Koch recommended the administration of 1 gram (15 grains) of the drug every sixth and seventh, seventh and eighth, eighth and ninth, or ninth and tenth days, according to the severity of the malarial infections present, while Ziemann and Nocht recommended the administration of 1 gram (15 grains) of the drug every fourth day, giving it in four equally divided doses.

It is my belief that the prophylactic doses of quinine recommended by Celli, Sergeant, Ziemann, and Nocht are too small to prevent infection in many cases, and that experience has shown that in the prophylaxis of malaria a daily dose of 1 gram (15 grains) is necessary in order to prevent infection. The entire dose may be administered at one time, upon retiring, or half of the dose may be administered in the morning and the other half upon retiring at night. It is probably better to take the entire dose at night, but the relative value of the two methods of administration has not been determined. It should be distinctly understood that the dosage of quinine recommended above to prevent malarial infection in healthy individuals has nothing whatever in common with the dosage to be employed in preventing relapses of malarial infection or in the treatment of "carriers" of the malaria plasmodia.

As a prophylactic quinine must be administered by the mouth. When given in this way it may be administered in the form of a solution, in pills, tablets, capsules, wafers, troches, or confections. If possible, it should always be given in solution, except to persons with very sensitive stomachs, in which case it is better to use capsules or tablets. Tablets are often hard and insoluble, but when good soluble tablets can be secured this is a convenient form of administration. For children the chocolate-coated tannate of quinine troche is an excellent way in which to administer a drug that one usually has great difficulty in giving a child.

Quinine in solution possesses the advantage that it is more quickly and easily absorbed, with less irritation to the stomach, and that it is the cheapest way in which the drug can be administered. The solution is prepared by dissolving 65 centigrams of quinine sulphate in 8 cubic centimetres (2 drams) of water, to which two drops of concentrated hydrochloric acid have been added. A large amount of the solution can be prepared at one time, as it keeps well, and can be dispensed as needed.

In order to be effective, the administration of quinine as a prophylactic should be under the personal supervision of a health officer, and in the military service, of a medical officer. When this method of prophylaxis is being used in large numbers of men, as in military troops, the duty of overseeing the administration of the drug should never be delegated by the medical officer to any subordinate, but should be considered as one of the



most important *personal* duties that he is called upon to perform. Most of the reported failures of quinine prophylaxis in troops have been due to the relegation of this duty to careless subordinates, with the result that the method has been condemned without a fair trial. I believe that there is no instance of record in which at least 1 gram (15 grains) of quinine has been administered daily, under the personal supervision of a medical officer, who actually saw that the drug was swallowed, which was followed by failure, except in rare individual cases, to protect from malaria.

The administration of smaller doses of quinine than that recommended, *i.e.*, 1 gram (15 grains) daily, in the prophylaxis of malaria, should not be favored, for the reason that the smaller doses are inefficient and will not prevent malarial infection if exposure is continued for any length of time. Where there are many "carriers" of the plasmodia, and many anopheline mosquitoes, even a daily dose of 1 gram (15 grains) of quinine will be found inefficient in preventing infection, and, under such conditions, a daily dose of 1.3 grams (20 grains) of quinine should be administered for prophylactic purposes.

**Treatment of Initial Infections with the Plasmodia and of "Carriers" of the Plasmodia.**—If one wishes to prevent the infection of mosquitoes with the malaria plasmodia, and eventually the infection of man, the proper treatment of initial malarial infections, and of patients who have gametes in their blood, is of the very greatest importance. It is well known that after a malarial infection has persisted in man for from eight days to two weeks, provided insufficient quinine has been administered to kill all the plasmodia, there develop in the blood certain forms intended to undergo their development in the mosquito, known as gametes, and which, if ingested by the insect, eventually render it infective to man. It is evident that if these forms could be destroyed before reaching the mosquito, the plasmodia could not be transmitted to man. It follows, therefore, that if quinine can prevent the formation of gametes, and it can, every patient presenting these forms of the malaria plasmodia in the blood, provided he has been under the care of a physician, is an evidence of the improper treatment of his infection by the physician, unless the gametes were present when he was first seen, having developed as the result of a latent or unrecognized infection. As a matter of fact, the vast importance of the proper treatment of the infected in the prophylaxis of malaria is not even now realized by the medical profession and by some health officers, for there is nothing more common, in many malarial regions, than to see gamete carriers going about their daily occupations without having received any advice regarding quinine treatment, and wholly ignorant that they are a serious danger to the people of the community in which they reside. Neither has the profession realized the importance of so treating their malarial patients that gametes will not develop.

In any thorough campaign against the malaria plasmodia the discovery and treatment of *latent infections*, the *treatment of gamete carriers*, and the proper *treatment of initial and recurrent malarial infections*, are fully as important as other methods of malaria prophylaxis. As a matter of fact, the prevention of "carriers" of any infection, and the treatment of those who have become carriers, is one of the most important functions of preventive medicine, while the discovery of latent infections and their treatment would appear to be the first step in the prevention of any disease, and this is especially true of the fevers caused by the malaria plasmodia.

*The Discovery and Treatment of Latent Infections.* By a latent malarial infection we understand one in which the plasmodia may be demonstrated in the peripheral blood, but in which no clinical symptoms of sufficient gravity to attract the attention of the infected individual are observed. The term includes not only those cases in which no symptoms of malaria have ever been observed, but also those cases in which the symptoms are in abeyance between relapses or recurrences.

It is well known that in all malarial regions a considerable proportion of individuals in apparently good health have malaria plasmodia in their peripheral blood, and that the natives of such regions possess an acquired immunity to the usual effects of the malarial toxin or toxins, although the plasmodia may develop in such individuals in sufficient numbers to be demonstrated in the peripheral blood. It has also been shown that insufficient treatment of malaria results in the production of gametes and a latent condition of the infection, and that many patients who are discharged from treatment as cured may show plasmodia for a long time in their blood before a relapse occurs.

*The proportion of latent infection varies greatly in different localities*, but it may be stated that such infections occur in a considerable percentage of both children and adults in any region where malaria plasmodia are endemic, and that the proper treatment of these infections is of the greatest importance in the prophylaxis of malaria in these regions. In the following tables the incidence of latent malarial infection was determined by the finding of the plasmodia in the peripheral blood, and the figures are not based upon the number of cases of enlarged spleen, which, while a valuable index of latent malaria in some regions, cannot be relied upon in others, owing to the presence of other infections which cause enlargement of that organ.

Koch (1900) was one of the first to call attention to the presence of latent malarial infections in natives of malarial regions, and he concluded, from his observations in Africa, that such infections were confined almost entirely to children, but further observations, by many investigators, have proven that such infections occur frequently in adults, and that the true

index of latent malaria in any region can only be determined by the examination of the blood of both adults and children.

The following tables give the results of several of the more important investigations of latent malaria in different localities, and furnish an excellent idea of the variation in the number of these infections in different regions.

*Table I. A. Plehn's (1903) Observations in Kamerun, West Africa.*

Age	No. Examined	No. Infected	Percentage
Under 2 years of age.	18	17	94
Between 2 and 5 years.	26	24	92
Between 5 and 10 years.	40	34	85
Adults.	43	26	60
Total.	127	101	44.4

*Table II. Olwig's (1903) Observations in Dutch East Africa.*

Age	No. Examined	No. Infected	Percentage
Under 1 year of age.	93	33	35.4
1 year to 5 years.	220	83	37.7
5 years to 15 years.	971	109	11.2
Adults.	650	105	16.1
Total.	1,934	330	17.0

*Table III. Craig's (1906) Observations at Camp Stotsenberg, Island of Luzon, Philippine Islands.*

Age	No. Examined	No. Infected	Percentage
1 to 5 years.	40	30	75
5 to 10 years.	54	20	37
10 to 15 years.	53	13	24.5
Adults.	45	28	62.2
Total.	192	91	47.3

*Table IV. Thomas's (1910) Observations in Manáos, Brazil.*

Age	No. Examined	No. Infected	Percentage
6 months to 2 years.	9	3	33.3
2 years to 5 years.	38	20	52.6
5 years to 8 years.	51	28	54.9
8 years to 10 years.	59	27	45.7
Total.	157	78	49.6

*Table V. Sergent's (1909) Observations in Algeria.*

Age	No. Examined	No. Infected	Percentage
1 to 5 years.	1,316	267	20.2
6 to 10 years.	1,360	326	23.9
11 to 15 years.	933	272	29.1
Adults.	2,471	722	33.3
Total.	6,080	1,587	26.1

*Table VI. Sorel's (1912) Observations on Ivory Coast, Africa.*

Age	No. Examined	No. Infected	Percentage
Under 1 year.	134	75	56
1 year to 5 years.	253	141	56
5 years to 15 years.	328	125	38
Adults.	275	110	43
Total.	990	451	44.4

The above tables demonstrate that latent malarial infections occur frequently in adults as well as in children, and similar figures have been secured by Bass (1919) in his examinations in Mississippi. The blood of 31,459 individuals was examined for the plasmodia, and of these 6,664 showed plasmodia in the blood, or 21.18 per cent. The rate of infection under 20 years of age was 23.56 per cent., while the rate over 20 years of age was 19.22 per cent. Of the 6,664 individuals showing plasmodia in their blood, 3,671, or 55.09 per cent., gave a history of a malarial attack within a year, while 2,993, or 44.91 per cent., gave a negative history of malaria.

The following table, compiled from the observations of others, and my own, gives a good composite picture of the incidence of malarial infection in most localities where the plasmodia are endemic and mosquitoes are numerous.

*Table VII. Consolidated Table Showing Prevalence of Latent Malarial Infections at Various Ages.*

Age	No. Examined	No. Infected	Percentage
1 to 5 years.	6,288	1,407	22.3
5 to 10 years.	5,305	1,279	24.1
10 to 15 years	3,480	966	27.7
Adults.	15,440	3,164	20.4
Total.	30,513	6,816	20.4

It may be stated that at least one-fifth of the inhabitants of malarial regions probably carry the plasmodia around in their blood without having noticeable symptoms of the infection, and that in many badly infected regions the percentage of latent infections in children runs close to 90 per cent., and of adults close to 60 per cent. Therefore, it is evident that latent malarial infection is of very great importance in the prophylaxis of the plasmodia, for unless these infections are discovered and properly treated the plasmodia will continue to infect mosquitoes, and the malarial fevers will continue to be prevalent.

The percentage of "carriers" of malaria among the latent cases varies considerably, but it may be safely stated, from my own observations, that from 40 to 50 per cent. of individuals who have such infections have



gametes in their blood and are "carriers" of malaria. The percentage will vary with the amount of quinine that the individual may have taken from time to time, but at least 40 per cent. of latent cases are "carriers," and undoubtedly a much larger percentage occurs in regions badly infested and where practically no treatment has been administered.

The *recognition of latent malarial infection* is possible by either a microscopical examination of the blood for the plasmodia or by the palpation of the spleen, which is generally enlarged. Of these methods the examination of the blood is the most accurate, in my opinion, for the splenic index, as it is called, while a most valuable guide to the prevalence of malaria in a locality, is useless in some regions, owing to the prevalence of other diseases, as kala-azar, in which the spleen is also enlarged. In addition, the enlargement of the spleen may simply mean past malarial infection, and does not, by any means, indicate that plasmodia are present in the blood, or that prophylactic treatment with quinine is necessary in order to prevent the development of gametes or to destroy them if they are present. Where blood examinations cannot be made the splenic index will have to be relied upon, but the only really scientific and accurate method of detecting latent malarial infections is by finding the plasmodia in the blood.

The proper treatment of latent infection is a most important prophylactic measure. Immediately upon the discovery of such infections treatment should be instituted, and the amount of quinine administered should vary with the number of plasmodia present and the presence or absence of gametes. The treatment of "gamete carriers" is considered later, and here only the treatment of latent infections in which no gametes are detected will be considered.

If gametes are not present the object is to prevent their development, and this is accomplished by the administration of proper doses of quinine. The vast majority of latent infections will eventually develop gametes, for while the development of these sexual forms may be long delayed, it undoubtedly occurs at some time in most of the latent cases unless proper treatment is administered. Fortunately, latent infections are amenable to treatment, and such individuals will not become infective to mosquitoes if we can prevent the development of the gametes. The following scheme of treatment will be found efficient in ridding the blood of plasmodia in these latent infections, and in curing the infection. While the amounts of quinine advocated may seem large, it has been found in practice that cures cannot be expected with less.

Every individual showing plasmodia in the blood, provided gametes are not present, should receive 2 grams (30 grains) of quinine daily until the plasmodia disappear from the peripheral blood. In most instances this will occur within four or five days, and often within two or three days.

The drug should be given in equally divided doses every three hours, and three such doses should be given, *i.e.*, each dose should contain 65 centigrams (10 grains) of quinine. It is convenient to give one dose in the morning, one at noon, and the other at night. After the plasmodia have disappeared from the blood quinine should continue to be given in daily doses of 1 gram (15 grains) for two weeks, and for a month afterward daily doses of 65 centigrams (10 grains) should be given. These amounts are best given at night upon retiring.

If this method of treating latent infections be faithfully carried out it will be found that latent malaria will disappear, and that none of the individuals so treated will become gamete carriers, and thus infective to mosquitoes, unless gametes should happen to develop within a day or two of commencing treatment. The time to treat latent malarial infections is before the development of gametes, because these forms are much more resistant to quinine than are the forms concerned in the human life-cycle of the plasmodia, and because the isolation and screening of the infected individual is not necessary if gametes are not present.

*The Discovery and Treatment of "Carriers" of the Plasmodia.* One of the most important and, until quite recently, one of the most neglected, prophylactic measures against the spread of malarial infection is the discovery and treatment of "carriers" of the plasmodia. It has already been stated that after a malarial infection has persisted for from a week to two weeks in man, forms of the plasmodia develop which are capable of further development in anopheline mosquitoes, and that these eventually render the insect infective to man. These forms are called *gametes*, and they can be easily detected in the blood of man by a microscopical examination of stained preparations. It is obvious that if these gamete carriers can be detected and the gametes killed, or prevented from developing further, the individuals harboring them will cease to be infective to mosquitoes, and malarial infection of man by these insects will be impossible. It has been shown that gamete carriers can be rendered harmless by proper treatment with quinine, and that it is feasible to employ the drug in this way in the prophylaxis of malarial fevers.

The treatment of gamete carriers depends upon their discovery, and this can only be done by a microscopical examination of the blood of both apparently healthy individuals in malarial regions as well as those suffering from malarial infection. Gametes are found not only in patients suffering from obvious symptoms of malaria, but also in those having a latent malarial infection, and it is believed that these forms are not introduced as such by the infecting mosquitoes, but develop during the life-cycle in man. The number of individuals infected with the plasmodia who will become gamete carriers will vary, of course, in different localities, owing to conditions favoring the persistence of the infection, the type of infec-

tion, the thoroughness with which treatment is carried out, and perhaps other conditions with which we are not familiar. In the æstivo-autumnal infections gametes may be rare in the peripheral blood but numerous in the capillaries of the internal viscera, especially the spleen, so that a microscopical examination of the peripheral blood may be misleading in this respect, a fact which should be remembered when apparent failures are observed in the treatment of such cases. Based upon personal experience, it is probably safe to say that at least 35 to 50 per cent. of individuals who have developed definite symptoms of malarial infection and who have had no treatment, or insufficient treatment, become gamete carriers, and that at least 40 per cent. of latent infections eventually develop gametes and become infective to mosquitoes.

It has been shown by Darling (1910) and Thompson (1912) that a certain number of gametes must be present in the peripheral blood of an individual in order to produce infection in the mosquito. Darling arrived at the conclusion, based upon experimental evidence, that the peripheral blood must contain at least 1 gamete per 500 leucocytes in order to be infective to the mosquito, and that individuals whose blood contains this number of gametes should be regarded as gamete carriers, and kept under treatment in a screened room until the number of gametes is reduced below this minimum. The number of gametes is easily ascertained by comparing the number counted in a stained blood-smear with the number of leucocytes counted.

There is still some difference of opinion regarding the action of quinine upon the gametes, but it is certain that after the gametes are fully developed they are very resistant to the drug. However, experience has shown that they do disappear in time if the drug is given in proper dosage, and this disappearance is probably due to the action of the drug upon the young intracorpuseular gametes and upon the asexual forms from which they develop. It has been shown by numerous investigators that it is possible to reduce the number of gametes in the peripheral blood by continued administration of quinine in large doses over a considerable period of time. Both Darling and Thompson demonstrated that the administration of large doses of quinine, over a period of about three weeks, results in the reduction of the number of gametes in the peripheral blood to a non-infectious minimum, *i.e.*, to less than 1 per 500 leucocytes, and recent work upon prophylactic treatment of gamete carriers has shown conclusively that proper treatment with quinine will render these carriers harmless.

The *treatment* of gamete carriers demands most careful supervision, and should always be controlled by frequent microscopical examinations of the blood and counts of the gametes. Any individual having more than 1 gamete to 500 leucocytes in his blood should be kept under treatment until the gametes disappear or, at least, until they are reduced below the num-

her mentioned. During treatment carriers should be kept in a screened room or beneath mosquito nets, owing to the fact that they are infectious to mosquitoes.

When gametes are present in large numbers, 2 grams (30 grains) of quinine will generally have to be administered daily for at least three weeks before the peripheral blood becomes non-infectious to the mosquito, but where the gametes are only slightly above 1 per 500 leucocytes 1.3 grams (20 grains) of quinine daily will be found sufficient if given over the same period of time in daily doses. Some authorities, as Bass, have found smaller doses of quinine efficient in sterilizing gamete carriers, if continued over longer periods of time. A daily dose of 65 centigrams (10 grains) continued for eight weeks was found very efficient by Bass in anti-malarial work in Mississippi, and the carriers were not screened, as it was impossible to do so in the majority of the individuals treated. Where this method of prophylaxis is applied to whole communities screening will, of course, have to be omitted for economical and social reasons, as most of the carriers will have latent infections, and will not be in hospital or confined to their homes.

*The proper treatment of initial or recurrent malarial infections* is most important in prophylaxis. It has been shown that the forms of the plasmodia that infect mosquitoes do not develop until several days after the infection has produced symptoms, so that the best time to begin malaria prophylaxis is with the treatment of every acute infection, and if this is done, gametes will not develop, and the patient will not become a carrier of malaria. Every gamete carrier is evidence of either improper treatment or of no treatment, and if of improper treatment, an evidence of ignorance or carelessness on the part of the attending physician. It is a sad fact, but a true one, that a very large proportion of the malaria present in most localities is directly due to the improper treatment of malarial infections coming under the direct care of medical practitioners, because of the common practice of regarding such infections as cured when the symptoms disappear, the patient being allowed to resume his work without any direction regarding the continued use of quinine.

The first requisite in the proper treatment of an acute malarial infection is a correct diagnosis, and this should always be made by finding the plasmodia in the blood. Not only should the diagnosis be made microscopically, but treatment should be controlled by frequent blood examinations, and no patient should be allowed to resume his occupation until the peripheral blood is free from plasmodia. If the blood contains only the forms occurring in the human life-cycle of the plasmodium it will be found that the parasites will disappear within a few days after beginning quinine, together with all symptoms of the infection, and the patient may be returned to duty with directions concerning the continuance of quinine,



but if gametes are present it will mean from two to three weeks of treatment with the dosage of quinine already mentioned before they will be reduced to a non-infectious minimum.

The *dosage of quinine* in the treatment of initial and recurrent malarial infections will vary somewhat with the type and severity of the infection, but during the acute attack, while symptoms are present, 2 grams (30 grains) of quinine should be given daily in divided doses as long as symptoms persist, and thereafter 65 centigrams (10 grains) of the drug should be administered either in one dose at night or 32 centigrams (5 grains) morning and evening for a period of at least three months. In æstivo-autumnal infections it may be necessary to give 2.65 grams (40 grains) of quinine daily until symptoms disappear, but it is seldom necessary to exceed this dose in the vast majority of malarial infections. Relapses should be treated in the same manner, and it is well to remember that tertian infections, *i.e.*, infections with *Plasmodium vivax*, while requiring smaller doses of quinine to control the symptoms, are most resistant to treatment as regards the cure of the infection, and frequently quinine has to be continued for a longer time in tertian infections than in either quartan or æstivo-autumnal infections.

**The Results of Prophylaxis.**—It is unnecessary here to discuss the remarkable results that have followed the application of prophylactic methods designed to destroy the mosquitoes responsible for the transmission of the malaria plasmodia. The practical eradication of malaria from cities like Havana, Cuba, Panama City, and others, has been accomplished largely by means of this method of prophylaxis, and the remarkable record made in our camps during the World War in malaria prophylaxis was practically entirely due to the destruction of the breeding-places of anopheline mosquitoes.

The prophylaxis of malaria by protecting man from the bites of mosquitoes has been followed by marked success in many localities. In Sardinia, Procaccini (1900) reduced the number of cases of malaria among soldiers from 70 per cent. to 57 per cent., in one season, by screening the barracks in which the men lived, and later the percentage was reduced to less than 20 per cent. In Formosa, Tzuzuki (1902) tested the efficiency of screening by selecting 115 soldiers of a battalion stationed at Kirun, a most malarial locality, and furnishing them with screened barracks, the remainder of the battalion living in unscreened barracks. The 115 men were thus protected from September 21 to December 8, the malarial season, and not a case of malaria developed among them, while in the same time, among 750 soldiers not so protected, there developed 319 cases of malaria. The men were confined in the screened barracks from half an hour before sunset to half an hour before sunrise during this time, and wore head-nets and gloves when on duty at night. Orenstein (1912)

relates a most interesting example of the value of screening in the settlements of Gatun and New Gatun, in the Canal Zone. Gatun had a population of approximately 4,500 residing in screened quarters, while New Gatun, having a population of about 5,000, had no screened quarters. With this exception the surroundings were identical so far as chances for malarial infection were concerned. Observations extending over three years showed that the malarial incidence of Gatun and New Gatun was as 2 is to 3, the following being the yearly incidence in each settlement:

Year	Gatun, Per Cent.	New Gatun, Per Cent.
1909	5.35	10.04
1910	5.37	9.21
1911	8.75	12.59

Orenstein states that properly screened dwellings will reduce by at least one-third the malaria incidence in localities where malaria is endemic.

The value of quinine prophylaxis has been proven in numerous instances, although there are some who are so prejudiced against it as to deny it any value, but to an unprejudiced observer it is very evident that it is a valuable method when properly applied. The results obtained by Celli (1913) in Italy are most illuminating in this respect. In the Italian army quinine prophylaxis has been in operation since 1903. In 1902 the incidence of malaria in the army was 27.44 per cent. In 1904, two years after beginning quinine prophylaxis, it had dropped to 19.21 per cent., and in 1911, the percentage of cases in the entire army was only 4.9 per cent., and this despite the fact that the troops were often stationed in the most malarious parts of the country. In the penal colony of Castiades, 92 per cent. of the population of over 700 suffered from malaria in 1904. In 1905 prophylactic quinine was instituted, and in 1906 the malaria incidence had dropped to 48 per cent., and by 1911 had dropped to 6 per cent. Regarding this reduction in malaria from 92 per cent. to 6 per cent., Celli says: "It was the prophylactic administration of quinine alone that produced the miracle of diminishing malaria." In the whole of Italy the mortality from malaria decreased from 21,000 deaths in 1887 to about 3,000 in 1908, quinine prophylaxis having been established about 1905, and Celli says: "Beyond doubt the greatest and most persistent decrease of mortality from malaria in Italy was due to the increased consumption of quinine."

The recent work of Bass, in the Mississippi Delta, has shown the great value of quinine prophylaxis, especially when used in the treatment of initial and latent infections and for the sterilization of gamete carriers. This region is one where it is not economically feasible to control mosquito breeding, so that protection from the bites of mosquitoes and quinine

prophylaxis are the only methods of prophylaxis available. An intensive campaign was begun by Bass in 1918 in a 100 square mile area in Mississippi, the blood of the inhabitants being examined for the plasmodia, and all found infected being placed under treatment, which consisted of the administration of 65 centigrams (10 grains) of quinine daily to adults, and proportionate doses to children. By the end of 1919 the incidence of malaria was only 13.2 per cent. among residents of the 100 square mile area who had been treated in 1918, as compared with the incidence of 40.2 per cent. that obtained among the same people before treatment, a reduction of 67.2 per cent.

The observation of Major Bascom, U. S. Army, at Ebert Field, Arkansas, showed conclusively that screening and sterilization of carriers by quinine furnishes almost absolute protection against malaria. In 1917, before these measures were instituted, there were four deaths from malaria in the control area, while the incidence of the disease was 29 per cent., or 522 cases. In 1918, after the institution of screening and the treatment of carriers, there were no deaths from malaria, and only one case of the disease developed. The sterilization of carriers was accomplished by the daily administration of 65 centigrams (10 grains) of quinine continued for 30 days. It was impossible, in this locality, to destroy the breeding-places of mosquitoes.

The *relative value* of the various prophylactic methods which have been described are well shown in the data collected by Castellani during the World War. These data concerned four different camps, all of which were located in endemic malarial regions which were similar as regards the intensity of the infection. In Camp No. 1, no anti-malarial measures were employed, and practically 100 per cent. of the officers and men developed malarial fevers. In Camp No. 2, quinine prophylaxis alone was employed; and in this camp 45 per cent. of the personnel developed malaria, a protection rate of 35 per cent. being obtained. In Camp No. 3, anti-mosquito measures alone were employed, and 25 per cent. of the personnel developed malaria, a protection rate of 75 per cent. In Camp No. 4, both anti-mosquito measures and quinine prophylaxis were employed, and in this camp only 6 per cent. of the personnel developed malaria, a protection rate for the men of 94 per cent., and for the officers of 97 per cent.

The above results show conclusively that the fullest measure of success in the prophylaxis of the malaria plasmodia is attained if all available methods of prophylaxis are utilized, and for this reason one should never depend upon any one method unless forced by local conditions to do so. It should also be remembered that the success of any method depends upon the manner of its application, and this is especially true of quinine prophylaxis in all of its varied applications, whether in the prevention of initial



infections, in the treatment of latent, initial, and recurrent infections, or in the sterilization of the malaria carrier.

Under certain conditions some one method of prophylaxis may be much more valuable than another. For instance, Wenyon (1921) found that in Macedonia the protection of the English troops from the bite of mosquitoes by mosquito nets was a most valuable method of prophylaxis and he states that "mosquito nets did more to prevent infection than all the other methods of prevention of malaria together." However, the value of this method was due to local conditions that made other prophylactic methods less valuable than they usually are in normal conditions.

The prophylaxis of the malaria plasmodia should be studied from a broad standpoint, and not from the point of view of the enthusiast in any one method of prophylaxis. While it may be possible, in rare instances, to prevent malaria by using only one method of prophylaxis, as, for instance, the destruction of the breeding-places of anopheline mosquitoes, in the vast majority of instances one must use practically every method which has been advocated, and this is the policy that should be followed if success is to be assured.

**THE DIAGNOSIS OF THE MALARIA PLASMODIA.**—The diagnosis of malarial infection depends upon the demonstration of the malaria plasmodia in the blood of the infected individual, and it is only in this way that an accurate and scientific diagnosis can be made. The plasmodia may be demonstrated without much difficulty, in the vast majority of infections, by a careful examination of stained preparations of the peripheral blood of the patient, but in æstivo-autumnal infection, and in latent infections with any of the malaria plasmodia, several blood-smears may have to be examined before plasmodia are encountered. It has not been my experience, which has covered many years and the examination of thousands of blood-smears from malarial patients, that cases of infection occur in which the plasmodia are so few in number that it is impossible to demonstrate them by an examination of stained blood-smears, if symptoms of the infection are present, and I have yet to see a case of malaria severe enough to give rise to even slight symptoms in which it was impossible to demonstrate the plasmodia in the peripheral blood, if repeated examination were made, provided quinine had not been administered. On the other hand, I have observed malaria plasmodia in hundreds of cases of latent malarial infection in which no symptoms were present at the time of examination.

**Methods of Examination for the Plasmodia.**—**A. Apparatus Required.**—The apparatus required for the examination of the blood for the malaria plasmodia is simple, occupies little space, and may, if necessary, be transported to the patient's bedside, and the examination made on the premises. It consists of a good compound microscope, equipped with a one-twelfth-inch oil-immersion objective, a bottle of immersion oil, microscopic slides



and cover-glasses, two or three medicine droppers, a bottle of Wright's stain, and a bottle of distilled water. The one-twelfth oil-immersion objective is absolutely necessary, for while the larger forms of the tertian and quartan plasmodia and the *gametes* of all of the species of plasmodia may be seen with lower-power lenses, the small "ring-forms" can be seen distinctly only with the one-twelfth immersion lens.

**B. Preparation of Stained Blood-smears.**—The ear lobe or tip of the finger is cleaned with alcohol, dried, and a puncture made with a needle or blood lancet. The first drop or two of blood is wiped off, and then a very small drop of blood is collected upon the end of a clean microscopic slide, and as quickly as possible the end of another slide is brought in contact with the drop, either before or behind it, and the blood allowed to spread along the applied edge. As soon as this occurs the applied slide is pushed or drawn gently along the slide containing the drop of blood, and when this is properly done, a thin even smear of the blood is obtained. Several such smears should be made, so that if the first slide examined is negative others may be examined.

After the blood-smear has dried it is stained with a stain that will differentiate the malaria plasmodia. The Appendix contains directions for preparing several such stains, but I have found that the Wright stain is most useful in staining the plasmodia, as it is easily prepared, simple in operation, and uniformly excellent preparations are obtained with it.

A few drops of the stain are placed upon the blood-smear and allowed to remain for from two to four minutes, and enough of the stain should be used to allow for evaporation, for if this occurs the preparations will be worthless. At the end of from two to four minutes, at which time fixation is completed, enough distilled water is added drop by drop to cause a slight metallic scum to form upon the surface of the mixture. The stain is then allowed to act for five minutes, the specimens then washed thoroughly in distilled water and examined when dried, no cover-glass being necessary. The final washing is important, as this removes the precipitate that invariably forms during the process of staining, and also aids in the differentiation of the staining reactions of the plasmodia and the blood cells. The washing should be continued until the specimen has a delicate pink or pinkish-brown hue. Thoroughly boiled and cooled water may be used instead of distilled water if the latter cannot be obtained.

**C. Staining Reactions of Plasmodia and Blood Cells.**—The *staining reactions of the malaria plasmodia* have already been described, but it may be repeated here that the cytoplasm of the plasmodia stains a robin's egg blue with Wright's stain, while the chromatin of the nucleus stains a ruby red or violet color. The pigment usually appears greenish in *Plasmodium vivax* and *Plasmodium vivax minutum*, and greenish black or almost black in *Plasmodium malariae* and the æstivo-autumnal plasmodia.

The *staining reactions of the blood cells* are as follows. The red blood corpuscles are stained a pink or salmon color; the polynuclear leucocytes show a violet nucleus with unstained cytoplasm in which are multitudes of light-pink granules; the mononuclear leucocytes and lymphocytes have a dark ruby-red or violet nucleus, while the cytoplasm is stained a bright blue; the eosinophiles have a light-blue or a bluish-violet nucleus, while the cytoplasm is filled with bright pink or red granules; the mast-cells have purple-black granules and a dark-violet nucleus; while the blood-plates consist of a round or oval mass of red granules surrounded, in well-stained specimens, by a small amount of very light-blue-stained cytoplasm.

**D. Objects in Stained Blood-smears that May be Mistaken for Plasmodia.**—*Blood Platelets.* There is no object in the blood so frequently mistaken for malaria plasmodia as the blood platelets, especially in stained preparations. If a platelet happens to lie upon a red corpuscle, as it frequently does, the resemblance to a young schizont is quite marked, but the mistake can be easily prevented by remembering that any malaria plasmodium the size of a blood platelet is always a so-called “ring-form,” consisting of a distinctly blue-stained ring of cytoplasm, having at some portion of the periphery one, or at most, two, well-defined dark-red or violet dots of chromatin, or, in the case of the *gametes*, a similar dot of chromatin within the “ring.” The blood platelet consists of a more or less densely stained reddish mass of fine chromatin granules, often irregular in shape, with a poorly stained, generally very indistinct bluish cytoplasm surrounding the chromatin mass. A little practice should enable any one to differentiate the blood platelets from the ring-forms or gametes of the malaria plasmodia. A mass of blood platelets upon a red blood corpuscle is more confusing, but there should be no difficulty in distinguishing it from a plasmodium. Masses of blood platelets have been mistaken for sporulating plasmodia, but such a mistake could only be made by one entirely unfamiliar with the appearance of the blood elements in stained preparations.

*Flaws* in the microscopic slide or cover-glass filled with precipitated stain have been mistaken for malaria plasmodia, but if the structure of the plasmodia be remembered such a mistake is impossible.

*Leucocytes* have been mistaken for malaria plasmodia, but such a mistake could only be made by the merest tyro in blood examinations, and the same is true of mistaking yeast cells, pollens of various kinds, nucleated erythrocytes, or degenerated leucocytes, for the malaria plasmodia. One should be thoroughly familiar with the morphology of both normal and pathological blood before undertaking to diagnose the plasmodia in malarial infections.

**The Diagnosis of the Species of Malaria Plasmodia.**—In stained preparations of blood it is not difficult to make a differential diagnosis between

the various species of malaria plasmodia, and the following summary gives the essential differential features between the different species.

*Plasmodium vivax*. (The tertian plasmodium.) All stages of the human cycle of development are present in the peripheral blood. The young "ring-forms" cannot be distinguished from those of other species, except by an expert, but older forms are always present, which are differentiated by their bizarre shape. After pigment has developed the plasmodia become very amoeboid, the "ring" form is lost, and they are distinguished by their irregular and very bizarre shape. At all stages in the development the tertian plasmodium is larger than any of the other malaria plasmodia. The sporulating forms are easily recognized by their very large size, the red cells containing them being from two to three, or even four, times the size of the normal cells, and by the number of spores, or merozoites, which varies from 12 to 24, generally averaging from 16 to 20.

The red blood corpuscle infected with *Plasmodium vivax* is invariably enlarged, even during the very early stages in the development of the plasmodium, and after the development of pigment the infected erythrocyte is markedly larger than the normal erythrocytes. The cytoplasm of the infected erythrocyte undergoes a peculiar form of degeneration evidenced by the occurrence of red-stained granules throughout the cytoplasm, which are known as Schüffner's dots. Any enlarged red blood corpuscle containing a malaria plasmodium, and which shows these dots, is infected with *Plasmodium vivax*, and this is a very important and easily demonstrated diagnostic point. It should be remembered, however, that not every erythrocyte infected with *Plasmodium vivax* shows Schüffner's dots, and that many infections occur in which these dots are not observed, but which are due to *Plasmodium vivax*. *Plasmodium vivax minutum* also causes a degeneration in the erythrocyte, accompanied by the development of Schüffner's dots, but the infected erythrocyte is not enlarged.

*Plasmodium vivax minutum*. This is a rare species, and is differentiated from other malaria plasmodia by the occurrence of all stages of the human life-cycle in the peripheral blood; by the lack of enlargement of the infected erythrocyte but by marked deformity in the shape of the latter; by the occurrence of Schüffner's dots in the cytoplasm of the infected erythrocytes; by the bizarre shape of the young pigmented plasmodia; and by the number of spores or merozoites, which vary from 6 to 12 in number, thus resembling *Plasmodium malariae*. This species might be easily confused with the latter species, but the occurrence of Schüffner's dots in the infected erythrocytes and the absence of "band-forms" should serve to distinguish them, while it differs from *Plasmodium vivax* in the lack of enlargement of the infected erythrocyte and the small number of merozoites.



*Plasmodium malariae*. (The quartan plasmodium.) In stained blood-smears this species is distinguished from *Plasmodium vivax* by the lack of enlargement of the infected erythrocyte; the smaller size of the plasmodium throughout its cycle of development in man; and the absence of Schüffner's dots in the cytoplasm of the infected erythrocyte. All forms of the human life-cycle occur in the peripheral blood, and the number of spores, or merozoites, varies from 6 to 12, the average being between 6 and 8. From *Plasmodium vivax minutum* this species is differentiated by the absence of the characteristic "band-forms" and the absence of Schüffner's dots in the infected erythrocyte.

In infections with *Plasmodium vivax*, *Plasmodium vivax minutum*, and *Plasmodium malariae*, all forms concerned in the human life-cycle, or schizogony, and the gametes, occur in the peripheral blood, a fact which at once serves to distinguish these species from *Plasmodium falciparum* and *Plasmodium falciparum quotidianum*, in which only the "ring-forms" and gametes usually occur in the peripheral blood. Another distinguishing feature is that in infection with the first three plasmodia the infected erythrocyte is practically entirely filled by the plasmodium when sporulating, while in infections with the two æstivo-autumnal plasmodia the infected erythrocyte is not filled by the plasmodium when fully developed.

*Plasmodium falciparum*. (The tertian æstivo-autumnal plasmodium.) In the vast majority of infections with this plasmodium only the "ring-forms" are found in the peripheral blood, and it is only in pernicious infections that large pigmented and sporulating forms may be found, and then only in small numbers. The "ring-form" of *Plasmodium falciparum* generally shows a marked enlargement at some portion of the periphery, giving it the so-called "signet-ring" appearance, but otherwise these forms do not differ from those of other species except in size. Another distinguishing feature of this plasmodium, and also of the quotidian æstivo-autumnal plasmodium, is the occurrence of crescentic-shaped gametes in the peripheral blood, the so-called "crescents." These do not occur in infection with *Plasmodium vivax*, *Plasmodium vivax minutum*, or *Plasmodium malariae*, as the gametes of all these species are circular in shape. The sporulating forms almost fill the infected erythrocyte, and contain from 10 to 30 merozoites, the average being from 18 to 24.

*Plasmodium falciparum quotidianum*. (The quotidian æstivo-autumnal plasmodium.) As in infections with *Plasmodium falciparum*, the "ring-forms" occur most frequently in the peripheral blood, except in pernicious infections, but small pigmented forms are generally present in scant numbers in most infections with this parasite. The ring-forms of this species are much smaller than those of any other species of malaria plasmodia, many of them measuring not more than 0.5 micron in diameter,



and are so minute that they are frequently overlooked, even in stained blood-smears. Not only are they much smaller than the "ring-forms" of *Plasmodium falciparum*, but differ greatly from them in morphology, the chromatin being much larger in amount, and instead of occurring as a dot, or at most, two dots, at some portion of the periphery of the "ring," it forms an elongated mass composing a considerable portion of the "ring," extending around it for a considerable portion of its circumference. The gametes of this species are crescentic in shape but much smaller than the crescentic gametes of *Plasmodium falciparum*. The sporulating forms fill only one-half or a little more of the infected erythrocyte, and the spores, or merozoites, number from 6 to 18, the average running between 12 and 14, the latter number being most frequently encountered.

In infections with both *Plasmodium falciparum* and *Plasmodium falciparum quotidianum* the infected erythrocyte is never enlarged, but is usually smaller than normal, especially in infections with the latter species.

**Important Differential Points in the Diagnosis of the Malaria Plasmodia in Stained Blood-smears.**—The following summary of the most important differential points in the differentiation of the various species of malaria plasmodia will be found of service in diagnosis.

1. *Plasmodium vivax*. 1. Comparatively large size of plasmodium after development of pigment. 2. Increased size of the infected erythrocyte and distortion in shape. 3. Presence of Schüffner's dots (eosinophilic granules) in the cytoplasm of the infected erythrocyte. 4. Number of merozoites, or spores, 12 to 24. 5. Presence of all stages of the human life-cycle in the peripheral blood. 6. Schizogony completed in 48 hours. 7. Gametes spherical.

2. *Plasmodium vivax minutum*. 1. Medium size of plasmodium after development of pigment. 2. Infected erythrocyte not enlarged but much distorted in shape, oval cells being frequently observed. 3. Presence of Schüffner's dots in a small proportion of the infected erythrocytes. 4. Number of merozoites, 6 to 12. 5. Presence of all stages of schizogony in the peripheral blood. 6. Schizogony completed in 48 hours. 7. Gametes spherical.

3. *Plasmodium malariae*. 1. Medium size of plasmodium after development of pigment. 2. No increase in the size of the infected red blood corpuscle and no distortion in shape. 3. Absence of Schüffner's dots in the infected erythrocyte. 4. Occurrence of the so-called "band-forms" or "ribbon-forms," consisting of a band-like mass of blue-stained cytoplasm stretching across the infected erythrocyte, and containing chromatin and pigment. 5. Number of merozoites, 6 to 12. 6. Presence of all stages of schizogony in the peripheral blood. 7. Schizogony completed in 72 hours. 8. Gametes spherical.

4. *Plasmodium falciparum*. 1. Comparatively small size, even when

fully developed. 2. Infected erythrocyte, generally slightly smaller than normal and never enlarged. 3. Small amount of pigment. 4. Presence of basophilic granules in infected erythrocyte in some cases. 5. Number of merozoites, 10 to 30. 6. The sporulating forms fill about three-quarters of the infected erythrocyte. 7. Crescentic gametes or "crescents." 8. Only "ring-forms," very small pigmented forms, and gametes occur in the peripheral blood. 9. Schizogony completed in approximately 48 hours.

5. *Plasmodium falciparum quotidianum*. 1. Very minute size, especially of the "ring-forms." 2. Infected erythrocytes smaller than normal, crenated and often distorted in shape. 3. Smaller amount of pigment than in any of the other species of malaria plasmodia. 4. Basophilic granulation of infected erythrocyte sometimes present. 5. Number of merozoites, or spores, 6 to 18. 6. Only "ring-forms," very small pigmented forms, and gametes occur in the peripheral blood, except in pernicious cases. 7. Very small crescentic gametes, or "crescents." 8. The sporulating forms fill only about one-half of the infected erythrocyte. 9. Schizogony completed in 24 hours.

Attention to the differential features of the various malarial plasmodia given in the above summary should enable any one qualified in making microscopical examinations of the blood to make a diagnosis of the species of plasmodium present in any case of infection. In practice the plasmodia are numerous enough in the peripheral blood in the vast majority of infections to enable one to make a diagnosis after the examination of a single specimen, or at most, two specimens, although rare cases may be observed in which several blood-smears will have to be examined before the plasmodia are encountered. In such instances the use of the thick blood film is recommended, and directions for the preparation of such preparations will be found in the Appendix. In my own experience the thick blood film has not been found necessary, even in the examination of the blood of natives for latent malarial infection, except in a very few instances, and the use of this method is not recommended as a general procedure, both because it is unnecessary and, to be really useful, requires the services of an expert technician. Ordinary blood-smears, if well stained, and carefully and patiently examined, will give results in practice that are all that is required in the diagnosis of the plasmodia.

Because of the importance of differentiating the two æstivo-autumnal malaria plasmodia, *Plasmodium falciparum* and *Plasmodium falciparum quotidianum*, the following table of the important differential points, based upon personal observations of many hundreds of blood-smears containing these plasmodia, has been prepared, and will be found useful in the diagnosis of the plasmodia.

*Differential Diagnosis of the Æstivo-autumnal Malaria Plasmodia in Stained Preparations*

Period of Development	<i>Plasmodium Falciparum</i>	<i>Plasmodium Falciparum</i> <i>Quotidianum</i>
1. Length of cycle in man.	Forty-eight hours.	Twenty-four hours.
2. Earliest intracorpuseular stage.	Ring-form. Average diameter, 1.5 microns.	Minute oval body. Average diameter, 0.5 micron.
3. Morphology of ring-form.		
a. Size.	1.5 to 3.5 microns in diameter.	0.5 to 1 micron in diameter.
b. Cytoplasm.	Well-defined. Relatively large in amount.	Poorly defined. Relatively small in amount.
c. Chromatin.	Relatively small in amount. Composed of one or two spherical dots.	Relatively large in amount, composed of irregular or semi-lunar masses, forming a large portion of the ring-form.
d. Pigment.	Present. Fine grains in expanded portion of ring.	Never present in the ring-form.
e. Effect on erythrocyte.	Slightly reduced in size.	Reduced in size. Distorted in shape frequently. Peculiar hole-like appearance of portion enclosed by ring.
4. Morphology of pigmented and pre-sporulating forms.		
a. Size.	1.5 to 6 microns in diameter.	1 to 3 microns in diameter.
b. Cytoplasm.	Large in amount. Well-defined.	Small in amount and poorly defined.
c. Chromatin.	Relatively small in amount.	Relatively large in amount.
d. Pigment.	Small granules and irregular clumps.	Smaller in amount. One or two solid blocks.
e. Effect on erythrocyte.	Reduced in size.	Reduced in size and distorted in shape.
5. Morphology of sporulating forms.		
a. Size.	5 to 6.5 microns in diameter.	3 to 3.5 microns in diameter.
b. Cytoplasm.	Large in amount. Well-defined.	Small in amount. Poorly defined.
c. Chromatin.	Each merozoite has a small round dot.	Each merozoite is almost all chromatin.
d. Pigment.	Irregular mass.	Solid, minute block.
e. Effect on erythrocyte.	Reduced in size. Sporulating plasmodium almost fills erythrocyte.	Reduced in size. Distorted in shape. Sporulating plasmodium fills only about one-half of erythrocyte.
f. Number of merozoites.	10 to 30.	6 to 18.

In the diagnosis of the æstivo-autumnal plasmodia it should be remembered that the young ring-forms of *Plasmodium falciparum quotidianum*

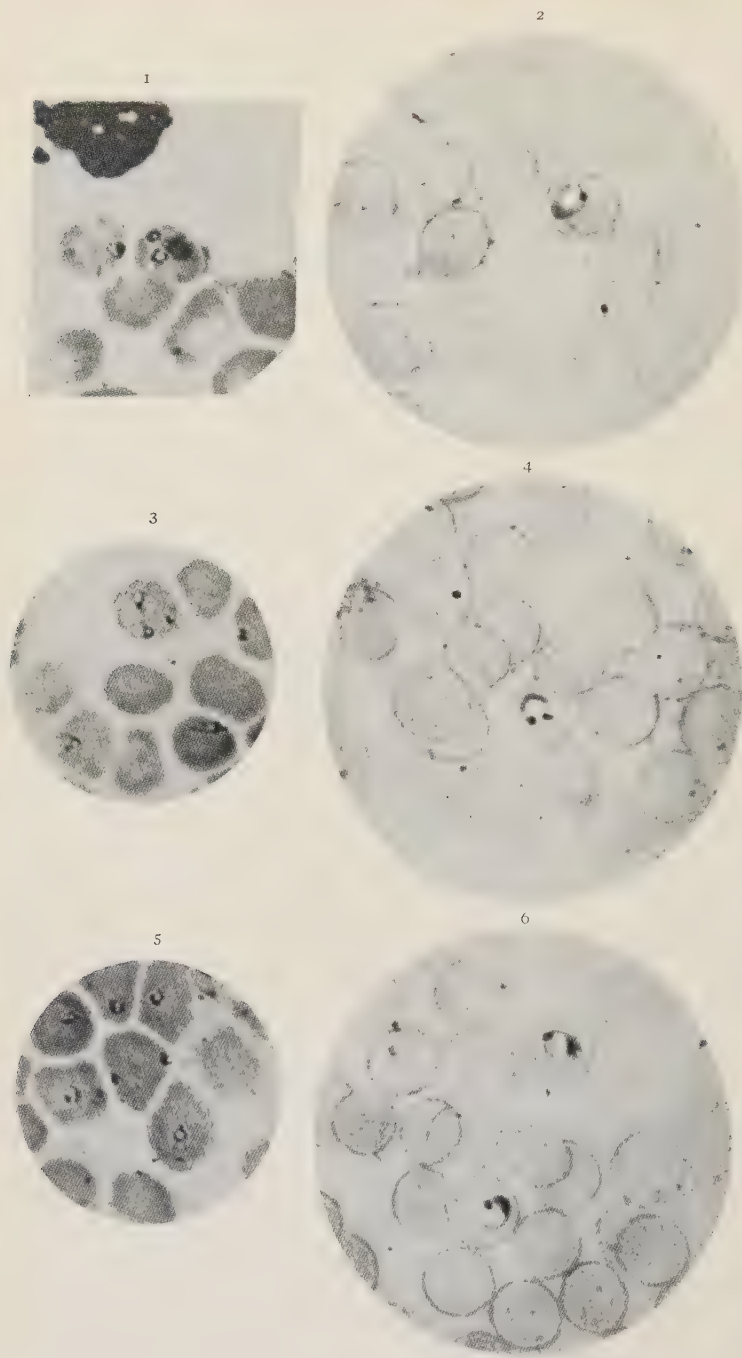


FIG. 87.—"Ring-forms" of *Plasmodium falciparum* and *Plasmodium falci-parum* quotidianum.  $\times 1,600$ . (From Army Medical School Collection. Photomicrographs.) Stained with Wright's stain. 1. *Plasmodium falciparum* quotidianum. "Ring-forms." Note relatively large amount of chromatin, one of the "rings" appearing to be composed almost entirely of this material. (In all the photomicrographs the very dark staining portion of the plasmodium represents the chromatin.) Note minute size of parasite.  $\times 1,600$ . 2. *Plasmodium falci-parum*. "Ring-form." Note dot of chromatin, and expanded portion of the "ring." Note large size of the ring compared with the ring-forms in figure 1.  $\times 1,600$ . 3. *Plasmodium falciparum* quotidianum. "Ring-forms." Note minute size and large amount of chromatin, comprising a large portion of the ring. Also "hole-like" appearance of portion of erythrocyte enclosed by the "ring-forms." Triple infection of one erythrocyte.  $\times 1,600$ . 4. *Plasmodium falci-parum*. "Ring-form." Note two small dots of chromatin, expanded portion of cytoplasm of the "ring," and the large size as compared with the "ring-forms" of the quotidian subspecies. The "ring-form" in this species fills almost as much of the erythrocyte as the three "ring-forms" of the quotidian parasite shown in figure 3.  $\times 1,600$ . 5. *Plasmodium falci-parum* quotidianum. "Ring-forms." Note minute size, and large amount of chromatin.  $\times 1,600$ . 6. *Plasmodium falci-parum*. Typical "ring-forms." Compare with "ring-forms" of quotidian plasmodium.  $\times 1,600$ .



are very minute, resembling much more closely pyroplasms than the ring-forms of the other malaria plasmodia, and that these forms are frequently overlooked even by trained microscopists, especially in unstained preparations of blood.

**Measures for Increasing the Number of Plasmodia in the Blood.—**

Various provocative methods, as they are called, have been advocated from time to time for increasing the number of plasmodia in the peripheral blood. The application of the X-ray or of ice to the abdomen over the area of the spleen has been found efficacious by some observers, but the injection of certain drugs has given the best results in the hands of most investigators. Of drugs, the hypodermic injection of ergotin, strychnine, and adrenalin have given the best results, especially adrenalin. Sarsen (1919), after a careful review of all of the provocative methods of diagnosis that have been tried, concluded that the subcutaneous administration of 1 mg. of adrenalin and the use of the ultra-violet ray over the splenic area, with physical exercise, were the best methods. On the other hand, Di Pace (1923) found that he secured the best results by the subcutaneous injection of 2 to 3 mgs. of strychnine nitrate, many doubtful cases showing numerous plasmodia in the peripheral blood after such injections. Dazzi (1919) found that the administration subcutaneously of 1 mg. of adrenalin was by far the best method for forcing the plasmodia into the peripheral blood. He found that the organisms began to appear in the peripheral blood about 20 minutes after the administration of the drug, and that the maximum number was reached about one hour after the injection. The plasmodia disappeared from the blood after 24 hours from the time of administration of the adrenalin.

The consensus of opinion appears to be that the administration of adrenalin in one-milligram doses, subcutaneously, is an efficient, if not the most efficient, of the provocative methods of diagnosis, and in certain doubtful cases it will be found most useful. However, it should not be forgotten that the careful examination of several stained films of the peripheral blood obtained without the employment of any provocative method is generally rewarded with success, and it has been very seldom that I have found it necessary to resort to such methods.

**Complement Fixation.**—An interesting method of diagnosis in malaria, from the scientific standpoint, is by complement fixation. Thompson (1919), using an antigen prepared by extracting the blood of patients rich in plasmodia, has been able to demonstrate that complement fixation will occur in the blood serum of malarial patients, but that it is not specific for the various species of plasmodia, as he found that *Plasmodium falciparum* antigen gave positive reactions with the blood serum of patients suffering from infection with *Plasmodium vivax*, and that *Plasmodium vivax* antigen gave positive reactions with the blood serum of patients

infected with *Plasmodium falciparum*. This method of diagnosis for malaria is not, at present, on a practical basis, but it may become a valuable method as the result of further research.

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## CHAPTER XVII

### THE SARCOSPORIDIA

The SARCOSPORIDIA belong to the NEOSPORIDIA, a group of PROTOZOA that form spores during the trophic stage of the life-cycle, amœbulæ arising from the spores. The SARCOSPORIDIA are not important as parasites of man, but some species cause severe and fatal infections in some of the lower animals.

**History and Nomenclature.**—The first observations upon sarcosporidia were made by Miescher (1843), who described white, filamentous structures, visible to the naked eye, in the muscles of mice. These structures ran parallel with the voluntary muscle fibres, and became known as "Miescher's tubes." The tube-like structures were enclosed in a membrane which was divided into compartments, in which were numerous rod-like or kidney-shaped bodies, as well as smaller spheroidal bodies. Miescher and others considered these bodies to be due to pathological changes in the muscles, and their parasitic nature was unrecognized until several years later, through the studies of Rainey, Leuckart, Manz, and Lindemann.

The SARCOSPORIDIA are parasites of vertebrates, and the genus *Sarcocystis* contains the species that are of greatest interest in animal pathology. Species of *Sarcocystis* have been described in the horse, in cattle, pigs, sheep, monkeys, opossums, antelopes, in domestic fowls, and in numerous species of birds. Reptiles, as the gecko and wall-lizard, have also been found infected.

Infection of man with *Sarcocystis* has been recorded by several observers, and it is probable that man is more frequently infected than is believed, as the symptoms produced by the infection are not diagnostic, and the infection is generally discovered at the autopsy table.

The most important species of *Sarcocystis* occurring in vertebrates are the following:

- Sarcocystis bertrani*, Doflein, 1901, in the horse.
- S. blanchardi*, Doflein, 1901, in cattle.
- S. colii*, Fantham, 1913, in the mouse-bird.
- S. darlingi*, Brumpt, 1913, in the opossum.
- S. hueti*, Blanchard, 1885, in the seal.
- S. kortei*, Castellani and Chalmers, 1909, in the monkey.
- S. lindemanni*, Rivolta, 1878, in man.
- S. miescheriana*, Kuhn, 1865, in the pig.
- S. muris*, Blanchard, 1885, in mice.
- S. rileyi*, Stiles, 1893, in ducks.
- S. tenella*, Railliet, 1886, in sheep.

**Morphology.**—The descriptions of the morphology, life-cycle, and method of infection of the SARCOSPORIDIA are incomplete, and much work remains to be accomplished before our knowledge regarding these parasites is in a satisfactory condition. Much of our knowledge of the SARCOSPORIDIA has been derived from the study of *Sarcocystis muris*, which

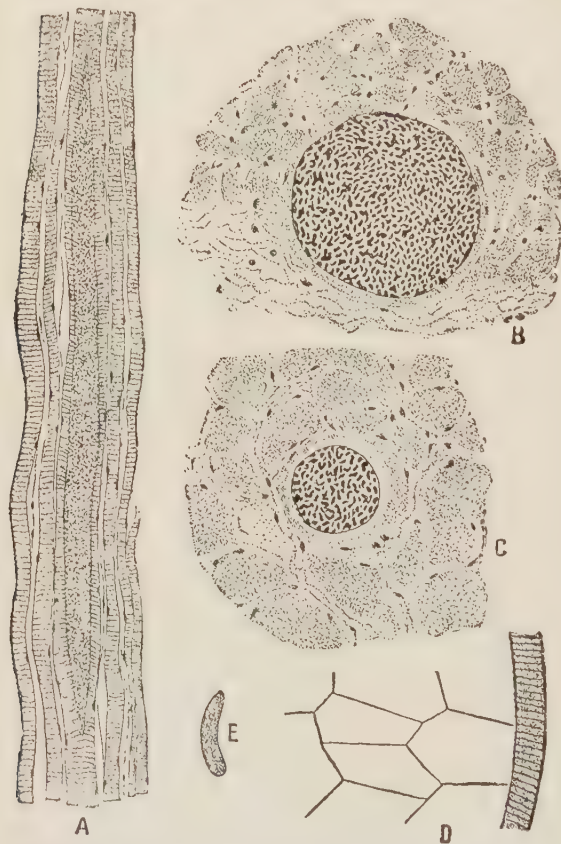


FIG. 88.—Sarcosporidia in vocal cords of man. A. Longitudinal section showing tubule filled with spores situated in a muscle fibre. B and C. Transverse sections of muscle fibres and tubule. D. Outer wall of parasite. E. Spore. (After Brumpt and Baraban and Saint-Remy.)

causes a fatal disease in mice. If these animals are fed upon the muscular tissue of mice infected with this parasite the sickle-shaped spores that are contained in the tubular structures are liberated in the intestine and penetrate into the lymph spaces. While passing through the intestinal epithelium to reach the lymph spaces the sickle-shaped spores become oval in shape, and division occurs by mitosis. From the lymph spaces the spores reach the muscular tissues, and the tubular structures, or Miescher's tubes, are formed, producing a bulging in the muscle fibres. The tubes are divided into separate compartments by prolongations from the membrane which encloses them, and in these compartments round, oval,

elongated, or sickle-shaped spores are produced. These spores are often referred to as Rainey's corpuscles. The tubes measure as much as 25 mm. in length, while the spores measure from 12 to 16 microns in length and from 4 to 9 microns in width. A toxin, called sarcocystin, discovered by Pfeiffer (1891), is secreted by *Sarcocystis muris*, which aids the parasite in penetrating the intestinal epithelium through its cytolytic action.

The fully developed SARCOSPORIDIA are cylindrical, elongated, or fusiform bodies, with rounded ends, enclosed in a distinct membrane and con-



taining multitudes of spores. These bodies are called sarcocysts, or Miescher's tubes, and occur especially in the muscles of the larynx, œsophagus, diaphragm, chest, and abdomen. Other muscles may be infected, as the heart muscle and those of the extremities, but not so frequently as those first named.

**Life-history and Method of Infection.**—The life-history of the SARCOSPORIDIA has already been described, so far as it is known. The natural method of infection has not been determined. Theobald Smith (1901) demonstrated that mice could be infected with *Sarcocystis muris* by feeding them the muscular tissue of infected mice, but this is probably not the natural method of infection in ruminant animals, even though it may be in mice. Negre (1910) fed mice upon the fæces of infected mice and produced infection, and Negri (1910) and Darling (1909) infected guinea-pigs by feeding them the tissues of infected mice. Erdmann (1914) infected mice with *Sarcocystis tenella* by feeding them the muscular tissue of infected sheep, thus proving that the sarcosporidian of the sheep could be transmitted to mice. It is probable that infection occurs naturally through the intestinal canal, and, as the fæces of mice infected with *Sarcocystis muris* are experimentally infective, fæcal contamination of grazing grounds may be the chief factor in the transmission of the sarcosporidia peculiar to ruminants, while contamination of food by the fæces may be the source of infection in other animals.

**Sarcosporidia as Parasites of Man.**—In 1868, Lindemann described bodies, occurring in the heart muscle of a man who had died of dropsy, which resembled sarcosporidia, but he regarded them as gregarines. In 1878, Rivolta reviewed the descriptions of Lindemann, and considered that the bodies were sarcosporidia, and named the species *Sarcocystis lindemanni*. There is still some doubt as to the nature of the bodies described by Lindemann, and the first well-authenticated instance of the infection of man with a sarcosporidian is that reported by Kartulis, in 1893, who found Miescher's tubes in the muscles of a Soudanese who died of multiple abscesses of the liver and abdominal muscles.

In 1894, Baraban and St. Remy found sarcosporidia in the laryngeal muscles of a criminal who had been executed. The parasites varied in length from 150 to 1,600 microns and in breadth from 77 to 168 microns. Blanchard named this parasite *Miescheria muris*, but it may have been identical with *Sarcocystis tenella*, of the sheep.

Vuillemin (1902) found a sarcosporidian in the muscles of a man dying from tuberculosis which he regarded as identical with *Sarcocystis tenella*, and Darling (1909) found sarcosporidia resembling *Sarcocystis muris* in the biceps of a negro from Barbados. This investigator has more recently (1919) reported a second case of infection in a native of the Federated Malay States. The observations of Cone (1921) regard-

ing the occurrence of a sarcosporidian in bone lesions in a child await confirmation.

It is still uncertain whether any species of SARCOSPORIDIA is peculiar to man, but the evidence available is against such a supposition. It is probable that, under certain conditions, man may become infected with certain species peculiar to some of the lower animals, but the method of infection is unknown.

#### RHINOSPORIDIUM SEEBERI, Wernicke, 1903.

This parasite was discovered in nasal polypi in natives of India, and was long known under the name *Rhinosporidium kinealyi*. It was believed, until recently, to be a protozoan parasite belonging to the order HAPLOSPORIDIA, but the researches of Ashworth (1923) apparently prove that it belongs to the fungi, and Ashworth classes it among the PHYCOMYCETES.

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## CHAPTER XVIII

THE CILIATES OF MAN. *BALANTIDIUM COLI*. *BALANTIDIUM MINUTUM*. *NYCTOTHERUS FABA*. DOUBTFUL SPECIES. DIAGNOSIS.

The Phylum of the PROTOZOA known as the CILIOPHORA contains a few species that are parasites of man, all belonging to the Class CILIATA, Order HETEROTRICHA. The CILIATA are often called INFUSORIA, and this is the name most frequently used in medical works.

The morphology of the CILIATA varies greatly in different genera and species, and the entire group is divided into four Orders, *i. e.*, HOLOTRICHA, HETEROTRICHA, HYPOTRICHA, and PERITRICHA. All of the ciliates of man belong to the HETEROTRICHA, and are the largest of the PROTOZOA occurring in the human host.

The body of a ciliate is covered with a cuticle furnished with innumerable apertures through which protrude the cilia with which the cuticle is covered externally. The body is bilaterally symmetrical, but in some species the shape constantly changes, due to the contractions of the body. In such species there is a hyaline ectoplasm and a granular-appearing endoplasm. The cilia are composed of ectoplasm and vary in appearance in different species. In most species the cilia are hair-like and delicate, but in some they may resemble thorns or hooks, the body presenting a remarkable appearance by reason of its surface being covered with these peculiar cilia.

The CILIATA are motile organisms, motility being rendered possible by the waving motion of the cilia. The cilia are also operative in the procurement of food, in some species, by causing currents in the fluid medium in which the organism lives, which direct the food particles into the mouth of the parasite.

A well-defined mouth is present in most of the CILIATA, called a peristome or cytostome. The mouth may be lined with cilia or undulating membranes may be present, which, by their movement, direct the food particles within it. Connected with the peristome there may be a pharyngeal cavity lined internally with cilia. An anal opening is present in some species, situated at the opposite end of the organism from the mouth or peristome.

The endoplasm, which forms the greater part of the parasite, is granular in appearance and contains two nuclei, a large nucleus which is called the macronucleus or meganucleus, and a smaller nucleus, which is called the micronucleus. The macronucleus presides over the somatic activities of the organism, while the micronucleus presides over the reproductive activities. The endoplasm may also contain one or two contractile vacuoles,

and numerous other vacuoles may be present which contain food particles or bacteria.

Reproduction in the CILIATA is by means of binary longitudinal division, or, after encystment, by multiple division. Conjugation occurs in most of the species, the conjugants lying side by side, and separating after conjugation is complete. This process in the CILIATA has been studied especially by Bütschi (1876), Maupas (1888), Hertwig (1889), and Calkins (1901), and their observations have shown that it is absolutely essential to the continued existence of the organisms. In the CILIATA conjugation occurs after repeated binary fissions of the organism, and results in a regeneration, or rejuvenescence, of the reproductive activities of the two conjugants. Very complicated changes occur in the micronucleus of the conjugants, consisting of repeated divisions of the micronuclei and the exchanging of the newly formed micronuclei by the conjugants, which eventually become the new micronucleus and macronucleus of each conjugant, the old macronucleus disintegrating and disappearing. When these nuclear changes are completed, or before, in some species, the conjugants separate. After the nuclear changes are complete reproduction occurs for several generations by binary fission, after which conjugation is repeated.

Encystment occurs in almost all species of the CILIATA when environmental conditions become unsuitable for reproduction by fission and conjugation. In all the species in which encystment occurs it is essentially a protective process, but in some species reproduction occurs within the cyst.

The ciliates that are parasitic in man belong to two genera, *Balantidium* and *Nyctotherus*, and are all parasites of the intestine. There are three species parasitic in the human intestine, *Balantidium coli*, *Balantidium minutum*, and *Nyctotherus faba*, and a few doubtful species.

Genus I. BALANTIDIUM, Claparède and Lachmann, 1858.

This genus was founded by Claparède and Lachmann, in 1858, to include certain parasitic ciliates characterized by an oval shape, coarse cilia, longitudinal striations of the cuticle, contractile vacuoles, a horseshoe- or kidney-shaped macronucleus, and reproduction by binary fission. Conjugation and encystment also occur in species belonging to this genus. The type species is *Balantidium entozoon* of the frog.

Two species belonging to this genus are parasitic in the intestine of man, *Balantidium coli* and *Balantidium minutum*.

Species I. BALANTIDIUM COLI (Malmsten, 1857), Stein, 1862.

Synonyms: *Paramæcium?* *coli*, Malmsten, 1857. *Plagiotoma coli*, Claparède and Lachmann, 1858. *Leucophrya coli*, Stein, 1860. *Holophrya coli*, Leuckart, 1863.

**History and Nomenclature.**—*Balantidium coli* is the common ciliate of the intestine of man and pigs, and is the largest protozoan parasite occurring in the intestinal canal. It was first described by Malmsten



(1857), but many of the older writers state that Leeuwenhoek was the discoverer of *Balantidium*, and some of the more recent text-books repeat this error. Dobell (1920) has shown conclusively that to Malmsten belongs the credit of first describing this organism, and his description has since been confirmed and added to by numerous investigators.

Malmsten first found the parasite in the fæces of two dysenteric patients, and Leuckart (1861) and Stein (1862) confirmed his observations, and found that this species was a common parasite of the intestine of pigs. The later observations of Strong (1904), Brumpt (1909), and Walker (1913) have added greatly to our knowledge of this parasite, and its morphology has been recently minutely studied by McDonald (1922), who has also described a new species occurring in the intestine of the pig which he has named *Balantidium suis*, and which has not been found in man.

Malmsten (1857) believed the parasite to be a paramœcium and called it *Paramœcium? coli*, but Stein (1860) placed it in the genus *Leucophrya*, and named it *Leucophrya coli*. Leuckart (1861) did not agree with Stein's interpretation, and suggested the name *Holophrya coli*, believing that it belonged in the genus *Holophrya*. Later Stein (1862) rightly transferred the parasite to the genus *Balantidium*, and the specific name of the organism became *Balantidium coli*.

**Morphology.**—The morphology of *Balantidium coli* has been very exhaustively studied, and is very characteristic. As it is a large parasite, in fact, the largest of the protozoa occurring in the human intestine, it is easily studied and recognized, and the fact that it is undoubtedly the cause of a severe type of dysentery renders this species of special interest to the physician.

*Balantidium coli* is oval in shape, but more pointed at the anterior end than at the posterior. The size is variously given by different observers. Malmsten (1857), in his original description, gave the length as from 60 to 100 microns and the breadth as from 50 to 70 microns. Solojew (1901) states that the length is 65 microns and the breadth 40 microns. V. Prowazek gives the length as from 52 to 71 microns and the breadth as from 40 to 58 microns. Dobell and O'Connor (1921) give the length as from 50 to 70 microns and the breadth as from 40 to 60 microns. Most of the specimens that I have studied have measured from 50 to 80 microns in length and from 40 to 70 microns in breadth, but I have repeatedly seen individuals of this species measuring over 100 microns in length, and others measuring less than 30 microns in length.

Dobell and O'Connor (1921) suggest that there may be a number of distinct races of the parasite which may be distinguished by their size, but with this opinion I cannot agree, for it has been my experience that large and small individuals occur invariably in every infection, which would

hardly be the case if they represented distinct races, unless there is always a combined infection with several races, and this is hardly probable.

The largest specimens of *Balantidium coli* are just visible to the naked eye, but could not be recognized without the aid of the microscope. In the living condition they appear slightly greenish in color, and the entire surface is covered with fine cilia arranged in longitudinal rows. There is a more or less well-defined division of the cytoplasm into an outer portion, or ectoplasm, which is very thin, and an inner portion, or endoplasm, which constitutes most of the body of the organism.

At the anterior, or more pointed, end there is a peristome leading into a distinct mouth, or cytostome, situated on the ventral side of the body, and appearing as a cleft in the cytoplasm. A tubular or spherical cavity, or gullet, sometimes called the œsophagus, leads from the mouth into the cytoplasm of the organism, and it is through this that food reaches the body of the parasite. Lying in the endoplasm there are numerous food vacuoles, and two contractile vacuoles, one large one situated near the anterior portion of the body, and the other, much smaller, situated posteriorly. The contractile vacuoles are not usually distinctly visible, and pulsate at regular intervals, the posterior contractile vacuole apparently emptying into a small tube connected with the surface at the posterior end through an opening called the anal opening or anus.

In addition to the structures that have been mentioned, the endoplasm contains a large kidney- or bean-shaped nucleus, the macronucleus, and a smaller nucleus, the micronucleus. The macronucleus may lie in any part of the endoplasm, but is generally situated more or less transversely near the middle of the body, and the micronucleus is located generally in contact with it.

In properly stained preparations all of the structures mentioned may be clearly distinguished, and the more detailed description of the organism which follows is based upon such preparations unless otherwise stated.

The entire body of *Balantidium coli* is covered by a thin resistant skin, or pellicle, which turns in at the anterior end and forms the lining of the peristome. The pellicle is longitudinally striated, this appearance being produced by the rows of cilia. Each cilium emerges from the ectoplasm through an aperture in the pellicle, and arises from a distinct granule in the ectoplasm. After emerging through the aperture in the pellicle the cilia become free upon the surface of the body. The pellicle is easily penetrated by fluids and vital stains, but is quite resistant to mechanical agencies. The body of the parasite is very plastic, and because of this it is able to penetrate between opposing obstacles or between the epithelial cells of the intestine with little difficulty.

The ectoplasm is directly beneath the pellicle, and, except at the anterior end, is very thin. At the anterior end there is a triangular area composed

of ectoplasm and covered with cilia, which is known as the peristome. The ectoplasm is not sharply differentiated from the endoplasm, but gradually merges into the latter.

McDonald (1922), in his careful study of the minute morphology of *Balantidium coli*, states that the ectoplasm, except in the region of the peristome, is divided into alternate light and dark spiral bands. The dark bands are granular in appearance and project from the surface of the body, forming slight ridges in the latter.

The cilia, which cover the body of the organism, are short and delicate, and in stained preparations are usually invisible, as they do not retain the stain well when decolorizing methods are employed. Their situation in such specimens is best ascertained by observing the arrangements of the basal granules from which the cilia originate. These granules are situated directly beneath the pellicle, in the hyaline or bright band of the ectoplasm, and stain deep blue or black with hæmatoxylin stains. From each granule a ciliary root extends in a diagonal direction into the dark or granular band of the ectoplasm, and terminates in a secondary basal granule, according to McDonald.

The cilia lining the peristome and mouth are twice as long as those covering the body, and are used for the capture and propulsion of food into the mouth and gullet. These cilia also originate from basal granules in the ectoplasm just beneath the pellicle and, piercing the latter through tiny apertures, become free upon the surface of the peristome and mouth.

The cilia covering the body of the organism are concerned entirely in locomotion, the motility of the parasite being caused by the motion of the cilia. *Balantidium coli* is very actively motile, moving in a comparatively straight line forward, and rotating slowly upon its axis at the same time. If obstacles to its passage are met it endeavors to bore through them, and in the fæces organisms are often seen threading their way through material of quite dense consistence.

The peristome is a round or pear-shaped cavity at the anterior end of the body which terminates at its ventral end in the mouth, or cytostome, which, in turn, terminates in the gullet or œsophagus. Food is directed into the mouth by the cilia lining the peristome, and is then passed into the gullet, from which it enters the endoplasm in the form of a food vacuole.

The two contractile vacuoles, which apparently are situated in the endoplasm, are actually located in the ectoplasm, and are best studied in the living specimen. One is usually located anteriorly, and one posteriorly, and when ready to contract, project into the endoplasm. Pulsation occurs at regular intervals and, in my experience, the rapidity of pulsation depends very largely upon external conditions and upon the vitality of the organism. If kept at the body temperature, pulsation of the vacuoles is much more rapid than when the organisms are kept at lower temperatures,

and the pulsations become slower and slower as degeneration of the organism progresses, and finally cease. The pulsations may occur as often as every thirty seconds, or may be delayed for several minutes. The contractile vacuoles appear at first as two small drops of clear fluid, which gradually enlarge and approach one another until they apparently discharge into a third vacuole, which then contracts and discharges its contents through the pellicle, after which the original vacuoles reappear and the process is repeated.

The endoplasm of *Balantidium coli* is coarsely granular in appearance and contains the macronucleus, the micronucleus, and food vacuoles.

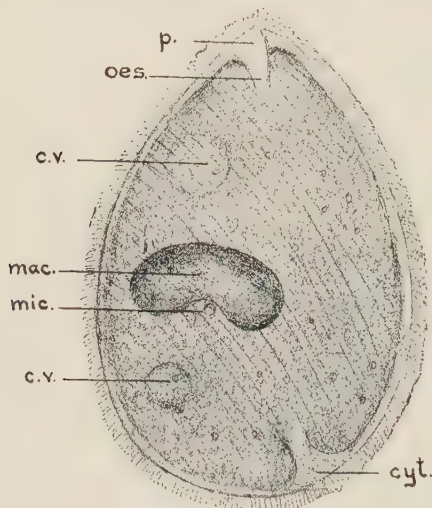


FIG. 89.—*Balantidium coli* from man. (After Hegner and Talliaferro.) C. V., contractile vacuole. Cyt., cytophyge. Mac., macronucleus. Mic., micronucleus. Oes., oesophagus. P., peristome.

The macronucleus is the most conspicuous object in the body of the parasite in stained specimens, and generally lies near the centre, but may be posterior or just beneath the cytostome, anteriorly. It is very large and usually kidney- or bean-shaped, but may be elongated or oval in shape. It has a nuclear membrane, which is generally invisible in deeply stained specimens, and is entirely filled with tightly packed masses of chromatin which stain so intensely that the entire nucleus often appears as a single, dense mass of undifferentiated material.

The micronucleus is often indistinguishable in stained preparations, owing to its minute size and close association with the macronucleus. It measures from 4 to 5 microns in length and is flattened upon one side, this side being in contact with the nuclear membrane of the macronucleus, in some instances, appearing to be partly embedded in the latter. It stains a deep blue or black with the hæmatoxylin stains. In my experience it is only in very carefully differentiated specimens that the micronucleus can be distinguished without difficulty by even the trained observer.

The endoplasm contains food vacuoles which may contain starch granules, red blood corpuscles, or bacteria. In degenerating specimens the endoplasm may be almost entirely filled with vacuoles containing bacteria.

The cysts of *Balantidium coli* may be found in freshly passed stools in some cases of infection, but not in all, unless repeated examinations are made covering long periods of time. They measure 45 to 65 microns in diameter and are spherical or oval in shape, and are by far the largest



protozoan cysts found in the human intestine. The cyst wall is double in outline, slightly greenish or yellowish in color, and the cyst, when first formed, contains a single balantidium which may be seen revolving within the cyst. Older cysts contain a mass of granular cytoplasm embedded in which is the macronucleus and a single contractile vacuole which empties its contents at regular intervals, while still older cysts contain simply a mass of cytoplasm and the macronucleus.

Cysts containing two organisms have been reported and described by several observers, but I have never encountered them in the material that I have examined.

**Habitat.**—*Balantidium coli* occurs in the large intestine of man, and is most numerous in the cæcum. It may be found living in the contents of the bowel, if they are of semi-fluid or fluid consistence, or actually within the tissue of the intestinal wall. Pathological studies have shown that this parasite may invade all of the coats of the large intestine and the neighboring lymph glands, as well as the blood-vessels and lymph channels of the intestine.

**Species Occurring in Lower Animals.**—*Balantidium coli* occurs not only in the intestine of man, but also in the intestine of pigs and monkeys. Leuckart (1863) first demonstrated its presence in pigs, and Brooks (1902–1903), during an investigation of an outbreak of dysentery among the ourang-outangs in the New York Zoological Park, discovered *Balantidium coli* in the stools of these animals. Noc (1908) and Brumpt (1909) have shown that this species also occurs in the intestine of monkeys belonging to the genus *Cynomolgus*.

Other parasitic species of *Balantidium* are found widely distributed in nature in hosts extending from crustaceans to warm-blooded animals, the greatest number of species occurring in the *Amphibia*. No less than ten well-differentiated species occur in frogs, and several species have been described that are parasitic in marine worms and in turtles.

**Cultivation.**—I have been unable to find any record of the successful cultivation of *Balantidium coli*, but I have observed apparent multiplication of the organisms in a medium composed of equal parts of alkaline beef broth and normal salt solution, and the organisms remained alive in this mixture for several days, being numerous in the culture tubes for as long as one week.

**Life-history.**—The life-history of this species has been very thoroughly worked out, and we know that it has a motile, vegetative stage of existence and a cystic stage. The vegetative stage is passed within the lumen of the intestine or in the tissues of the intestinal wall and neighboring lymph glands. When conditions for this vegetative existence are unfavorable encystment occurs in the lumen of the intestine, and the cysts are passed in the fæces, where they will remain unchanged for long periods of time

if the fæces are kept in a moist condition. The cystic forms have never been found in the tissues.

The parasite feeds upon material present in the lumen of the intestine and upon tissue cells. Starch granules, red blood corpuscles, leucocytes, tissue cells, bacteria, and crystals of various kinds have all been found within the food vacuoles in the endoplasm of *Balantidium coli*. The food particles are taken in through the peristome and mouth, pass to the gullet or oesophagus, and then circulate through the body in the food vacuoles.

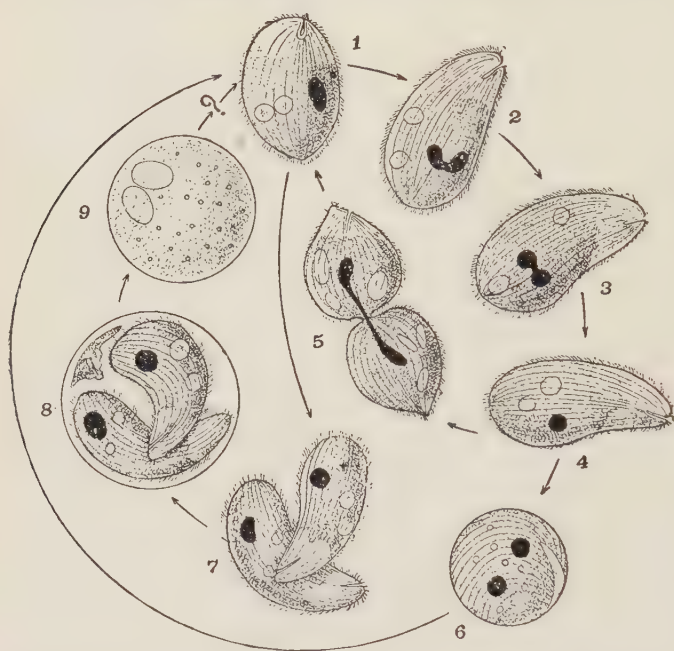


FIG. 90.—*Balantidium coli*. Diagram of supposed life-cycle. 1 to 5. Asexual reproduction by transverse division. 6. Cyst produced by one individual. 7 to 9. Conjugation and formation of a fusion cyst. (After Brumpt.)

The remains of the food, after digestion, are excreted, but in just what manner is still undecided. Some authorities claim that undigested material is voided through the opening at the posterior end of the organism which is called the anal opening.

Reproduction in the vegetative stage occurs by binary transverse division, the micronucleus first

dividing by mitosis followed by the division of the macronucleus, which is amitotic in character. After the division of the macronucleus the body divides into two individuals. Walker (1909) has described another form of division by budding, but I have never observed this in the material that I have examined. In degenerating organisms I have seen appearances that might be easily mistaken for budding, but these changes were undoubtedly due to degeneration of the cytoplasm.

Whether conjugation occurs in this species is still undecided. It has been described by some authors, and it is very probable that they are correct, and that conjugation is as important a stage in the life-cycle of this species as it is in that of many other species of *Balantidium*.

In this species encystment appears to be a purely protective process, and

reproduction does not occur within the cyst. As already noted, cysts containing two individuals have been described, but it is generally believed that this appearance is due to two organisms forming a cyst together, and not to a division of a single organism within the cyst. Further observation is necessary before a positive statement can be made regarding the origin of cysts containing two organisms. The cysts are the infective agents, and when swallowed liberate their contents in the large intestine, where the vegetative stage of development is resumed.

**Geographical Distribution.**—*Balantidium coli* is very widely distributed, and infections have been reported from Norway, Sweden, Russia, Finland, Germany, Austria, France, Holland, Serbia, and Italy, in Europe; from Siberia, China, Ceylon, in Asia; from Egypt, the Soudan, and Abyssinia, in Africa; from Cuba and from Porto Rico, and in South America from Brazil, Venezuela, and Honduras. Brug (1919) reported infections from Java, and Strong (1904) and others have reported cases in the Philippine Islands.

In the United States it has been found in man in Arkansas, California, Iowa, Louisiana, Minnesota, North Carolina, New York, and Oklahoma. No cases of human infection with this parasite have been reported from England, although it is a common parasite of the pig in the British Isles.

The only infections with *Balantidium coli* that I have personally observed have been in patients studied in the Philippine Islands, where the infections were contracted. It is a comparatively common parasite in native Filipinos in some districts, according to recent observations.

**Incidence of Infection.**—Although *Balantidium coli* is a very common parasite of the domestic pig, it is a rare parasite of man in most localities, and comparatively rare even in those regions where the greatest number of cases of infection have been reported. In the many thousands of examinations of stools made by numerous observers during the period of the World War few infections with this parasite were found. Kofoed (1921) and his co-workers, in the summary of their examinations of 2,300 American soldiers, do not report a single instance of infection with this parasite, and Mathews and Smith (1919), in 23,024 stool examinations in 4,068 English soldiers, most of whom were convalescent from dysentery, failed to find a single infection with *Balantidium coli*, according to their records.

During the past twenty-five years I have made many thousands of examinations of stools from many thousands of individuals, and have only encountered this parasite some half-dozen times, and never in any individual examined in the United States. Perhaps my experience has been unusual, but it is certainly good proof of the rarity with which it is encountered in routine examinations of stools. From my own experience, and that of many others, I have reached the conclusion that *Balantidium*

*coli* does not readily become parasitic in man, and that it is essentially a parasite of the pig.

**Method of Transmission.**—Man is infected by swallowing the cysts of *Balantidium coli* in contaminated food or drink. It is presumed that the cysts pass through the stomach and small intestine unchanged, and finally liberate the young balantidium in the large intestine. That this occurs largely in the cæcum is evidenced by the fact that this portion of the intestine always shows the greatest number of organisms.

The cysts remain unchanged in moist fæces for weeks, but are very slightly resistant to direct sunlight or desiccation. Therefore, they are not carried to food by the wind, but reach it through infected food handlers, or indirectly through the faulty disposal of sewage. The use of the excrement of pigs for fertilizer has been suggested as a common method of food contamination, but it is not believed that this method is of any great importance in the transmission of the parasite.

However, that man acquires his infection from the pig, in many instances, is shown by the fact that over 30 per cent. of the recorded cases of infection gave a history of direct contact with pigs or of eating pork products prepared in such a manner that the cysts of the parasite could have been present and viable at the time of ingestion.

Infection may also occur by direct transference of infected pigs' fæces to the mouth either through soiled hands or in slaughtering operations. Such an instance of infection is reported by Young and Walker (1918), in which an employee in a pork-packing factory, who handled pigs' guts, acquired a very heavy infection by getting pigs' fæces in his mouth repeatedly during the performance of his duties as a gut stripper. That such infections do not occur more frequently in packing-houses is remarkable, and certainly indicates that man possesses a considerable resistance to infection with *Balantidium coli*.

**Experimental Infection of Lower Animals.**—Casagrandi and Barbagallo (1896) produced a transient infection in cats with *Balantidium coli* of man, and Behrenroth (1913) also claims to have infected a cat with this parasite. Walker (1913) infected 2 of 4 monkeys with *Balantidium coli* from human fæces by rectal injections of the latter. Many negative experiments have been reported by different authorities, but, in most instances, it is probable that the material used for experimental feeding did not contain the cysts of the organism, and unless cysts were present, success could not be expected, as the vegetative forms are unable to withstand the acid of the gastric secretion, and are, therefore, unable to produce an infection when swallowed.

The transmission of *Balantidium coli* of the pig to monkeys has been accomplished by Brumpt (1919) and by Walker (1913), while Brumpt (1919) experimentally transmitted *Balantidium coli* from monkey to



monkey, and also *Balantidium coli* of the monkey to the pig. However, the experimental transmission of *Balantidium coli* of the pig and monkey to man has not been accomplished up to the present time. Here, again, we have proof of the resistance of man to infection with this parasite, even when large doses of the infective agent, the cysts, are administered. It would seem that certain conditions must be present in man before infection with this parasite is possible, for there is no reason to doubt that the *Balantidium* of the pig is identical with that which has been found in man.

**Relation to Disease.**—While *Balantidium coli* may live in the intestine of man without producing symptoms or pathological lesions, it is capable of causing severe symptoms of diarrhoea and dysentery, and of producing ulceration of the intestine. A distinct type of dysentery, known as balantidial dysentery, is recognized, in which the symptoms are very similar to those of amœbic dysentery, there being attacks of severe diarrhoea accompanied by bloody stools, alternating with periods of constipation. Unlike amœbic infection, involvement of the liver and liver abscess does not occur in infections with this parasite. Strong (1904), Bell and Couret (1910), Walker (1913), and Manlove (1917), have thoroughly studied and described the lesions produced by *Balantidium coli* in these cases, and my personal observations agree with theirs as regards the extensive ulcerative condition that this parasite may produce in the human intestine.

Walker (1913) believes that the parasite is capable of passing through healthy epithelium, and states that the process of penetration is not accompanied by necrosis or ulceration, but that the organism pushes its way between the epithelial cells or through them. In his experiments upon monkeys he invariably found the epithelium intact in the infected animals except for slight mechanical injuries, and that lesions of the epithelial layer produced by bacteria were not necessary before *Balantidium coli* could pass into the underlying tissues. Other authorities believe that the passage of these parasites into the tissues is not mechanical, but is rendered possible by the action of some cytolytic substance excreted by the organisms, which leads to the necrosis of the epithelial cells and the consequent breaking of the barrier furnished by the epithelium to the passage of the organisms. It cannot be said that the exact method of penetration of the balantidia into the tissues has been demonstrated, but I believe that the theory advocated by Walker is the most probable explanation.

Deep penetration of the organisms into the tissues is not necessary for the production of symptoms, for a diarrhoeal condition may be present before ulceration has occurred. In such instances the intestinal mucous membrane is hyperæmic, and may show superficial erosions and necrosis with small hæmorrhagic areas scattered between them.

When the parasites invade the mucous and submucous coats of the intestine they increase in number, and either produce abscesses or ulcera-

tions. The abscesses may be situated in the submucosa, extending even to the muscular layer, and may be covered with healthy-appearing mucous membrane, and contain a thick glairy material, often blood-stained and containing numerous balantidia, but otherwise sterile. The ulcers are round, oval, or irregular in shape, with undermined edges and floors covered with necrotic material and pus, and resemble very closely the ulcers produced by *Endamæba histolytica*. The mucous membrane between the ulcers may appear fairly normal or it may be swollen and show hæmorrhagic areas. As in amœbic infections, the ulcers may communicate with

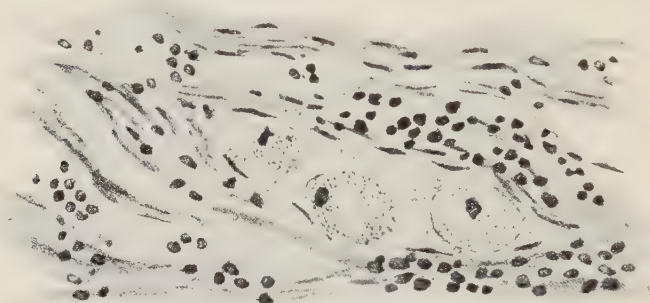


FIG. 91.—*Balantidium coli*. Three balantidia in a capillary of the submucosa in a case of balantidial dysentery. (After Dopter.)

one another through necrotic channels beneath the mucous membrane or upon the surface of the mucosa.

Sections of the infected tissues show round-celled infiltration, coagulation

necrosis in the walls of the abscesses and ulcers, hæmorrhages, and balantidia collected in or near the healthy tissue at the edge of the ulcers. The parasites may also be found within the capillaries and lymph channels of the infected tissue and in the neighboring lymphatic glands.

The pathology of balantidial dysentery is so much like that of amœbic dysentery that a differential diagnosis is generally impossible from a study of the lesions alone, and can only be made by finding the parasites in the abscesses or ulcerations.

In the monkey *Balantidium coli* produces similar lesions to those observed in man, and in these animals severe and even fatal dysentery may occur. In pigs, however, this parasite is generally a harmless commensal, but this is not always true, for Brumpt (1909) produced typical ulcerations in the pig by experimental infection, and Haughwout (1918) states that he has found *Balantidium coli* "in sections of pig intestine not only in the tissues but in the blood-vessels as well; in fact, the microscopic picture was similar to that seen in the case of human balantidiosis."

The evidence is conclusive that *Balantidium coli* is a pathogenic parasite of man, and while its presence in the intestine may not always be followed by symptoms, it, nevertheless, is capable of producing a severe form of dysentery, accompanied by very extensive and serious lesions in the intestinal coats.

Species II. *BALANTIDIUM MINUTUM*, Schaudinn, 1899.

This rare species of *Balantidium* was described by Schaudinn, in 1899, who found it in a single individual in Berlin.

The body is oval in shape with a rather pointed anterior end and a broad, rounded posterior end. It measures from 20 to 32 microns in length and from 14 to 20 microns in breadth. The entire body is covered with cilia that are longer and more delicate than those covering the body of *Balantidium coli*. The peristome extends as a long, well-defined groove from the anterior end backward to the middle of the body, or even beyond, and terminates in a gullet which merges into the endoplasm. The cilia lining the right lateral border of the peristome are similar to those covering the body, but those upon the left margin are considerably longer and stouter. The cilia originate in the ectoplasm and pass out through apertures in the cuticle, which is refractile in appearance.

The endoplasm is granular and contains numerous food vacuoles, a single contractile vacuole lying dorsally in the posterior end of the body, and the macronucleus and micronucleus. The macronucleus is situated at or very near the centre of the body, is spherical in shape, and measures 6 to 7 microns in diameter. It has a well-defined nuclear membrane, and in stained specimens a linen network may be distinguished, having upon it numerous irregular clumps of chromatin. The micronucleus is very small, being only about 1 micron in diameter, and lies in contact with the nuclear membrane of the macronucleus, and in front of the latter.

Reproduction in this species occurs by binary transverse division. Schaudinn did not observe conjugation, but states that cysts are formed which are oval in shape and contain a single organism.

*Balantidium minutum* was found by Schaudinn in the stools of a single patient suffering from diarrhoea. The patient was observed in Berlin, by Jacoby, and was a German. The parasites were only observed in the stools during attacks of diarrhoea, and disappeared when the attacks ceased. Schaudinn regarded the parasite as a harmless commensal.

Nothing is known as to the geographical distribution of *Balantidium minutum*, its method of transmission, or its relation to disease. It is evidently a very rare parasite of man, and Schaudinn's description has never been confirmed.

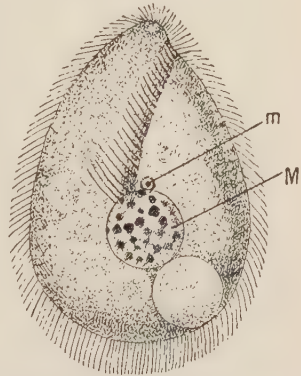


FIG. 92.—*Balantidium minutum* from man. M, macronucleus; m, micronucleus.  $\times 1,500$ . (From Brumpt, after Schaudinn.)

## Genus II. NYCTOTHERUS, Leidy, 1849.

Protozoa belonging to this genus are characterized by a kidney or bean shape; a peristome extending along the concave side of the body to about the middle; a single contractile vacuole situated at the posterior end of the body; and a spherical macronucleus situated at or near the centre of the body. Members of this genus are parasitic in amphibians, insects, and myriapods, and one species, *Nyctotherus faba*, is a parasite of the intestine of man.

## Species I. NYCTOTHERUS FABA, Schaudinn, 1899.

Schaudinn (1899) found this parasite in the stools of the same patient in which he discovered *Balantidium minutum*, and this is the only instance of infection with it that has been recorded with such accuracy that it can be accepted as authentic.

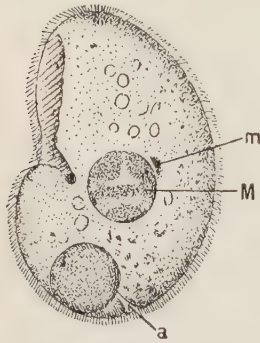


FIG. 93.—*Nyctotherus faba*.  
M, macronucleus; m, micro-  
nucleus; a, vacuole.  $\times 1,500$ .  
(From Brumpt, after  
Schaudinn.)

The body of *Nyctotherus faba* is bean-shaped and slightly flattened dorso-ventrally. It measures from 26 to 28 microns in length, 16 to 18 microns in breadth, and 10 to 12 microns in thickness. The body is covered with short delicate cilia, the peristome being lined with cilia that are much longer and stouter.

The peristome extends along the concave side of the organism from the anterior end to the middle of the body, where it terminates in a short gullet or œsophagus, obliquely placed, which empties into the endoplasm. The endoplasm contains a single large contractile vacuole, situated at the posterior end of the body, which expels its contents into a small duct or anus. The macronucleus is situated in the centre of the body, measures 6 to 7 microns in diameter, is spherical in shape, and contains four or five large masses of chromatin. It has a well-defined nuclear membrane. The micronucleus lies in contact with the macronucleus, measures from 1 to 1.5 microns in diameter, and is spherical or slightly oval in shape.

Schaudinn did not observe division or conjugation in this species, but states that oval cysts are formed.

Nothing is known regarding the life-history of *Nyctotherus faba*, its geographical distribution, method of transmission, or relation to disease. Other species of *Nyctotherus* are parasitic in frogs and insects, but none are pathogenic parasites, and Schaudinn did not regard *Nyctotherus faba* as a pathogen.

**Doubtful Genera and Species of Ciliates in Man.**—Several ciliates have been described as parasitic in man which must be considered as of doubtful status, and the most important of these will now be considered.



It should be remembered that free-living ciliates are common in nature, and that contamination of the stools of man frequently occurs with such ciliates. Many of the doubtful species that have been described have undoubtedly been of coprozoic origin, while others may be identical with one or another of the accepted species.

### 1. NYCTOTHERUS AFRICANA, Castellani, 1905.

This organism was found by Castellani in the stools of a native of Uganda who was suffering from sleeping sickness and severe diarrhœa. It measured 40 to 50 microns long and 36 to 40 microns broad. In shape it resembled an hour-glass with the constriction at the junction of the anterior and middle thirds of the body, which was covered with fine cilia, and contained a macro- and micronucleus and a single contractile vacuole. The macronucleus was large and spherical in shape, with the chromatin arranged in four equal granular masses separated by a poorly staining material free from chromatin. The micronucleus was small and placed in front of the macronucleus, and in close association with the latter. The peristome was situated in the posterior portion of the body. Nothing is known of the life-history, geographical distribution, method of transmission, or relation to disease of this organism, and it is very doubtful if it belongs in the genus *Nyctotherus*. It was found in the cæcum and other parts of the large intestine after death, as well as in the stools during life.

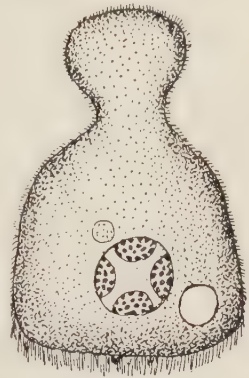


FIG. 94.—*Nyctotherus africana*. (After Castellani.)

### 2. NYCTOTHERUS GIGANTEUS, Krause, 1906.

This organism was found by Krause in the stools of a typhoid patient in Breslau. In his description Krause proposed the name *Balantidium coli giganteum* for it, but Doflein (1916), Fantham, Stevens, and Theobald (1916) and other authorities consider that it belongs in the genus *Nyctotherus*. Dobell and O'Connor (1921) do not agree with this interpretation of the status of the organism, and state that they are unable, from the descriptions available, to definitely determine its systematic position.

As described, *Nyctotherus giganteus* measures from 90 to 400 microns in length and 60 to 160 microns in breadth, being the largest ciliate described as parasitic in man. The body is oval in shape, narrow and rounded at the anterior end and broad and rounded posteriorly. It is covered with cilia and has a peristome situated laterally, and an anal opening on the posterior border. The macronucleus is large and bean-shaped, and

the micronucleus very small and spherical in shape. In addition to the nuclei the endoplasm contains one or two contractile vacuoles.

Krause states that he was able to keep the organism alive for five weeks in an alkaline medium kept at body temperature.

As this parasite has only been studied in the one case, and the description has never been confirmed, it must be regarded as a doubtful species.

**Other Doubtful Ciliates of Man.**—Guiart (1903) described a ciliate which he found in the stools of a patient with diarrhoea in France, and which he identified with *Chilodon dentatus*, and Manson and Sambon (1909) found a similar organism in the stools of a single patient, which they identified as *Chilodon uncinatus*. As organisms belonging to the genus *Chilodon* are common ciliates living in water and elsewhere, it is probable that in both the cases of Guiart and Manson and Sambon, the stools had become contaminated with such organisms and were not actually living in the intestine.

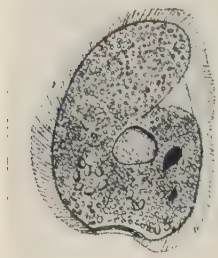


FIG. 95.—*Nyclotherus giganteus*. (After Krause.)

Martini (1910) found a ciliate in the stools of three patients suffering from dysentery, which he named *Uronema caudatum*. It was oval in shape, measured from 30 to 43 microns in length and from 11 to 16 microns in breadth, possessed a large peristome and a long delicate filament, situated at the posterior end. It was kept alive for several weeks in a mixture of faeces and normal salt solution kept at room temperature. Dobell and O'Connor (1921) believe this organism was really a *Cyclidium*, a species of which genus is a common free-living ciliate in water and infusions, and that Martini's material became contaminated with it in some manner.

In 1915, Barlow described a ciliate which he found in human faeces in Honduras, and which he regarded as a new variety of *Balantidium*, calling it *Balantidium coli*, variety *Hondurensis*. From his description I agree with Dobell and O'Connor (1921) in their opinion that it is probable that the organism was a free-living ciliate which had contaminated the stools after passage. The same interpretation is probably true of the so-called balantidia found by Marshall (1911) in the spleen of a patient who died of kala-azar; by Hinkelmann (1919) in the blood and urine of man; and by Sangiorgi (1919), who described a supposed variety of *Balantidium*, naming it *Balantidium coli*, sp. *Albanense*.

Faust and Wassell (1921), in a survey of the intestinal parasites of the Central Yangtze Valley, found a *Balantidium* too small to be *Balantidium coli* in two patients suffering from diarrhoea at Kuling. They did not have an opportunity to study the organisms, and have not published any description. It is possible that the species was *Balantidium minutum*.

**The Diagnosis of Ciliates.**—The diagnosis of the ciliates of man is made by finding the organisms in the fæces. For this purpose a small amount of the freshly passed stool is mounted and examined as already noted for the examination of stools for amœba, flagellates, and coccidia. The morphology of *Balantidium coli* is so distinctive, and the parasite is so large, that it could hardly be mistaken for anything else, either in the motile or encysted stage of development. The exact determination of the species of ciliate present, if there is any uncertainty, should be left to the trained protozoologist, as many mistakes have been made in this direction by untrained observers.

The greatest care should be taken not to confuse coprozoic ciliates with those parasitic in man, and in order to avoid such confusion, the stools should be passed directly into a dry and clean bed-pan and examined at once. If dilution is necessary, be sure that the distilled water or salt solution used is sterile, as free-living ciliates are often found in water and in distilled water that has been kept carelessly for several days.

The ciliates may be stained, after wet-fixation, with the hæmatoxylin stains, or dry smears may be stained with the Wright or Giemsa stain. Wet-fixation and the hæmatoxylin stains give the best results, but in order to bring out the cilia great care must be taken in differentiating. The general structure of ciliates is well shown in specimens stained with the Wright stain.

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## TECHNICAL APPENDIX

**Culture Media for Free-living Amœbæ.**—The culture medium that has been found most useful in the cultivation of free-living amœbæ from the stools was devised by Musgrave and Clegg, and will be found useful in the cultivation of many of the free-living species of amœbæ found in soil and water, as well as in the stools.

### *Musgrave and Clegg's Medium*

Agar .....	20.0 grams.
Sodium chloride .....	0.3– 0.5 gram.
Extract of beef .....	0.3– 0.5 gram.
Distilled water .....	1000 c.c.

This is prepared exactly as is ordinary nutrient agar, and is made 1 per cent. alkaline, using phenolphthalein as an indicator.

The free-living amœbæ develop upon this medium in symbiosis with the bacteria that may be present in the material examined and which form their chief food. The medium is poured into Petrie dishes, and after it has hardened the material to be cultured is smeared upon the surface, and the plates inverted and kept at a temperature of from 20 to 25° C., in a moist atmosphere.

*Walker's Medium.* Walker has used a medium which is identical with that of Musgrave and Clegg, with the exception that 2 c.c. of normal sodium hydroxide is added to each 100 c.c. of the medium. The reaction of this medium is neutral after sterilization, and he found that it was very satisfactory as a culture medium for free-living amœbæ.

**Culture Media for Parasitic Amœbæ.**—Cutler claims to have cultivated *Endamœba histolytica* on the following media, and to have subcultured them for several months, the amœbæ being pathogenic to cats, producing typical dysentery in these animals.

*Cutler's Media No. 1. Egg Medium.* The white and yolk of an egg is thoroughly shaken in a flask containing glass beads, and 300 c.c. of distilled water is added and the mixture again shaken. Bring the mixture slowly to the boiling point by heating over a water bath, and this temperature kept for half an hour, the mixture being agitated during the entire time. At the end of this time distribute the mixture in culture tubes, each containing 5 c.c. of the medium, and sterilize in the autoclave.

*Cutler's Medium No. 2. Blood-clot Medium.* One litre of water and 500 c.c. of human blood-clot is mixed, and the mixture boiled for one hour. It is then filtered, and to the filtrate is added 0.5 per cent. sodium chloride and 1 per cent. peptone. The whole is sterilized in an Arnold sterilizer for 20 minutes for three successive days.

With both of these media it is necessary to add a few drops of human blood before inoculating.

Cutler inoculated 5 c.c. of these media, to which a few drops of blood had been added, with a few loopfuls of bloody stool and incubated for 24 hours between 28–30° C., at the end of which time the cultures are examined for amœbæ. If successful, the amœbæ will be found in large numbers, and subcultures should be made daily or every two days.

*Boeck and Drbohlav's Media.* The following media have been found efficient by Boeck and Drbohlav for the cultivation of *Endamæba histolytica*, and by Drbohlav for the cultivation of *Endamæba gingivalis*.

*Medium No. 1. Locke-egg-serum, or L.E.S. Medium.* This medium is made as follows:

Four eggs are washed, brushed with alcohol and broken into a sterile flask containing glass beads. Fifty c.c. of Locke's solution (see page 537) are then added and the mixture broken up by shaking. Test tubes are then filled with sufficient to produce slants about 1 to 1.5 inches in length upon coagulation by heat. The tubes are slanted in an inspissator and heated at 70° C. until the egg mixture is solidified. They are then autoclaved at 15 pounds pressure for 20 minutes.

The tubes are then covered to a depth of 1 cm. above the egg slant with a mixture composed of 8 parts of sterile Locke's solution and 1 part of sterile inactivated human blood serum, and incubated to determine sterility.

*Medium No. 2. Locke-egg-albumin, or L.E.A. Medium.* Drbohlav improved upon the preceding medium by using crystallized egg albumin instead of human blood serum. A one per cent. solution of the crystallized egg albumin in Locke's solution was employed and this was sterilized by passage through a Berkefeld filter and then added to the tubes containing the egg slants as described above for the L.E.S. medium.

The initial reaction of the L.E.S. and L.E.A. media varied from P<sub>11</sub> 7.2 to 7.8 and needed no adjustment.

*Endamæba histolytica* was cultivated upon both of these media but the L.E.A. medium apparently gave the best results. A small amount of the material containing the amœbæ is inoculated beneath the fluid covering the slants and along the slant. The amœbæ in the cultures were found most commonly at the bottom of the culture tubes in the bacterial sediment and upon the lower one-half surface of the egg slant, and were most numerous on the second day after inoculation. Subcultures should be made every other day and the amœbæ are found in the cultures from three to six days, four or five days being the average life of the culture. In making subcultures a few drops of the sediment at the bottom of the culture are withdrawn with a sterile pipette and new tubes are inoculated by ejecting the material from the pipette near the bottom of the subculture.

The best results in cultivating *Endamoeba histolytica* were obtained when the tubes were cultured at 37° C.

The media mentioned above are also excellent for the cultivation of *Chilomastix mesnili*, *Trichomonas hominis*, *Blastocystis hominis* and *Tricercomonas intestinalis*.

### Culture Media for Intestinal Flagellates

Several culture media have been devised for the cultivation of intestinal flagellates, most of them having as a basis either Locke's or Ringer's solution.

#### Locke's Solution

Sodium chloride .....	0.9 gm.
Calcium chloride .....	0.024 gm.
Potassium chloride .....	0.042 gm.
Sodium bicarbonate .....	0.02 gm.
Distilled water .....	100.00 c.c.

Add 0.25 gm. of dextrose and sterilize.

#### Ringer's Solution

Sodium chloride .....	0.8 gm.
Calcium chloride .....	0.02 gm.
Potassium chloride .....	0.02 gm.
Sodium bicarbonate .....	0.02 gm.
Distilled water .....	100.00 c.c.

Add 0.1 gm. dextrose and sterilize.

#### Boeck's Medium

Locke's solution .....	4 parts
Human blood serum .....	1 part

After sterilizing the Locke's solution the human blood serum is mixed with it in the proportion given, and the medium distributed in culture tubes in 5 c.c. amounts. This medium was found very efficient in culturing *Chilomastix mesnili*. The tubes are kept at 37° C., in the incubator.

#### Hogue's Medium, No. 1

Locke's solution .....	200 c.c.
Egg, hen's .....	No. 1

The egg is broken into a flask containing glass beads and thoroughly shaken, after which the Locke's solution is added and the mixture heated over a water bath for 15 minutes, being kept in constant motion during this time. It is then filtered through cotton with a suction pump, tubed in 6 c.c. amounts, and autoclaved for 20 minutes at 15 pounds' pressure.

Hogue found this medium valuable in the cultivation of *Trichomonas hominis*.

#### Hogue's Medium, No. 2

Sodium chloride solution, 0.7 per cent. ....	600 c.c.
Egg white, hen's eggs .....	No. 6

The whites of six hen's eggs are placed in a flask containing glass beads and thoroughly shaken, after which the 0.7 per cent. sodium chloride solution is added. The mixture is heated over a water bath for 20 to 30 minutes, being constantly agitated while cooking, then passed through coarse cheese-cloth, and then filtered through cotton with a suction pump. Five c.c. of the filtrate is placed in each culture tube, the tubes are stoppered with cotton plugs and autoclaved for 20 minutes at 15 pounds' pressure.

This medium proved valuable in the hands of Hogue in the cultivation of *Trichomonas hominis* and *Embadomonas intestinalis*, and Hegner and Becker found that *Chilomastix mesnili*, *Trichomonas hominis*, *Embadomonas intestinalis*, and *Enteromonas hominis* could all be grown upon this medium. They recommend that the portion of fæcal material to be cultured be collected upon a toothpick, and when an amount about the size of an apple-seed has adhered, that the toothpick be dropped into the test tube containing the medium, and the whole incubated at about 36° C. The medium should be examined for flagellates in 24 hours, and the flagellates were found to be most numerous upon the surface of the medium.

#### Wenyon's Medium

Bacteriologic nutrient agar, 2 per cent. (P <sub>H</sub> 7.6) .....	30 c.c.
Sodium chloride solution, 0.85 per cent. (P <sub>H</sub> 7.6) .....	270 c.c.

The nutrient agar is added to the sodium chloride solution, and 10 c.c. of this mixture is placed in each culture tube and autoclaved at 120° C. When the tubes have cooled to 50° C., 20 drops of rabbit blood are placed in each tube, care being taken to see that the blood is kept sterile. The tubes should be incubated at 37° C. for 24 hours in order to test their sterility, and are then ready for use. They may be inoculated in the same manner as recommended for inoculating the tubes of Hogue's media.

Wenyon was able to cultivate *Embadomonas intestinalis* upon this medium, as well as a species of *Trichomonas* from a tortoise.

It would appear that the media of Boeck and of Hogue are the most useful ones in the cultivation of intestinal flagellates.

#### Noguchi and Ohira's Medium

Ascitic fluid .....	500 c.c.
Ringer's fluid .....	500 c.c.

Mix and distribute in tubes, adding a small piece of sterile tissue to each tube.

Noguchi and Ohira were successful in cultivating *Trichomonas buccalis* on this medium, and Pringault cultivated *Trichomonas hominis* on the same medium. Subcultures should be made daily if incubated at 37° C., and every 48 hours if kept at 22 to 27° C.

#### Kofoed and Swezy's Medium

Locke's fluid .....	9 parts
Rabbit or guinea-pig blood serum .....	1 part



The Locke's solution is sterilized, the blood serum inactivated, and both are mixed, sterilized, and distributed in culture tubes. Wagener used 10 per cent. of inactivated human blood serum instead of rabbit or guinea-pig serum with better results. These observers cultivated *Pentatrichomonas ardin delteili* upon this medium.

*Lynch's Medium*

Sodium chloride solution, 0.5 per cent. ....	10 parts
Human blood serum .....	1 part

The sodium chloride solution should be sterile, and the blood serum is added and thoroughly mixed. Upon this medium Lynch was successful in cultivating *Trichomonas vaginalis*.

### Culture Media for Blood and Tissue Flagellates

The following media have proven useful in the cultivation of the trypanosomes and leishmania, the most generally useful being the N.N.N. medium.

*Roger's Medium.* This medium consists of sodium citrate solution, and it was in this fluid that he was able to obtain the first cultures of *Leishmania donovani*. The method of preparation and use is as follows:

Prepare some normal salt solution and to it add about 8 per cent. of sodium citrate, dissolving thoroughly; if the resulting solution is not acid in reaction, it should be made slightly so by the addition of citric acid, after which it is sterilized in the autoclave. The solution is distributed in 1 c.c. quantities in small culture tubes and to each tube about 0.5 c.c. of the blood of the patient is added or a small amount of splenic pulp obtained by puncture. If organisms are scarce, it is best to add 1 c.c. of blood to 1 c.c. of the citrate solution. The cultures are incubated between 20 to 22° C. and the temperature should never go beyond 25° C. Great care should be taken that the material inoculated is sterile, except for the leishmania, and that the citrate solution is sterile, as bacterial growth quickly kills the leishmania.

#### *Young and Van Sant's Method of Cultivating Leishmania donovani*

1. Collect 10 c.c. of blood from a vein in a sterile syringe containing 2 c.c. of Locke's fluid and expel into a flask containing from 50 to 70 c.c. of the same solution.

2. Mix and divide between two 50 c.c. centrifuge tubes.

3. Centrifuge until the red cells are lightly packed at the bottom of the tube. This will require a speed of about 750 revolutions per minute for about 5 minutes.

4. Decant the cloudy supernatant liquid into another centrifuge tube and centrifuge for 5 minutes at about 1,375 revolutions per minute.

5. Plant the sediment so obtained in N.N.N. medium tubes made with rabbit blood and having a reaction of about  $P_H$  7.6 and incubate at  $22^\circ$  C. Flagellates appear in the cultures in from 10 to 12 days, provided sterility has been maintained throughout the process. This is stated to be a very efficient method of obtaining cultures of *Leishmania donovani* from the blood of infected individuals.

*Kligler's Medium*

Dextrose agar, 1 per cent. ....	10 parts
Normal salt solution .....	90 parts

The reaction of the medium should be between  $P_H$  7 and  $P_H$  7.6.

The medium is sterilized in the autoclave and distributed into culture tubes, each tube containing from 5 to 10 c.c. of the medium. Just before use, fresh sterile rabbit blood serum is added to each tube in the proportion of 1 part of blood serum to 10 parts of the medium.

This medium was found very efficient by Kligler in the cultivation of *Leishmania tropica*. The tubes are inoculated with material obtained from the edge of the sore by a capillary glass pipette, great care being taken to secure material from within the tissue at the edge of the ulcer and to avoid bacterial contamination. The cultures are incubated at a temperature between  $24$  and  $25^\circ$  C. Flagellates begin to appear in the cultures in from 3 to 4 days, but sometimes a week elapses before flagellates are found.

*Novy, MacNeal and Nicolle's Medium. The N.N.N. Medium*

This medium is probably the most generally used of any of the media for the cultivation of trypanosomes and *Leishmania*, and upon it many of the trypanosomes and *Leishmania donovani* grow well if it be properly prepared and used. The formula is as follows:

Agar .....	14 gm.
Sea salt .....	6 gm.
Distilled water .....	900 c.c.

Mix and bring to the boiling point and then distribute in tubes and sterilize in the autoclave.

In using, the tubed medium is melted and then cooled to  $48^\circ$  C., and to each tube is added one-third of its volume of sterile defibrinated rabbit's blood. This is well mixed with the medium by rotating the tube and then the tubes are slanted and allowed to cool. As the water of condensation at the bottom of the tube is where the organisms develop most numerous, it is well to slant the tubes and allow them to cool on ice, as this gives a greater quantity of water of condensation. After the agar is well set, the cotton plugs of the tubes should be covered with paraffin, so that evaporation will not occur. Before using, the tubes should be tested

for sterility by incubating at 37° C. for 24 hours. If sterile, they should be stored in an ice-box, if not used immediately.

*Row's Medium*

Defibrinated human or rabbit blood .....	10 c.c.
Distilled water, sterile .....	10 c.c.

When the blood is laked, add to each volume of laked blood 2 volumes of sterile 1.2 per cent. salt solution.

Row's medium has proven useful in the cultivation of *Leishmania donovani*.

*Thomas and Breinl's Medium*

This consists of chicken or veal infusion containing 1 to 2 per cent. salt, 2.5 to 3.5 per cent. agar, and 1 to 1.5 per cent. peptone.

This infusion is added to defibrinated rabbit's blood in the proportion of 2 to 1 or 3 to 2 parts.

*Trypanosoma gambiense* lived in this medium for 68 days, but no multiplication occurred and subcultures could not be obtained. The tubes are incubated at temperatures between 22 and 25° C.

*Gray and Tulloch's Medium*

This medium consists of the N.N.N. medium with defibrinated dog's blood added instead of rabbit's blood. Upon this medium Gray and Tulloch found that *Trypanosoma gambiense* multiplied, but they were unable to obtain subcultures. The cultures were incubated at temperatures between 22 and 25° C.

*Torres' Medium*

This medium consists of meat broth containing 7 per cent. of sodium chloride and 5 per cent. of peptone. The reaction should be PH 6.55 to 7.18.

Upon this medium Torres found that *Schizotrypanum cruzi* grew abundantly and multiplied at temperatures between 22 to 25° C. and that at 24° C. the organisms remained alive for 49 days.

**Culture Methods for Malaria Plasmodia**

*Bass and Johns' Method.* These observers were the first to cultivate the malaria plasmodia and their method is still the most generally useful of any employed for this purpose, and is given in their own words (*Am. Jour. Trop. Dis. and Preventive Med.*, I, 546, 1914).

For cultivating one generation of plasmodia, they proceed as follows:

"Blood is collected from the patient's vein at the bend of the elbow. If drawn with the syringe it is expelled directly into a defibrinating tube. The latter should be tilted to one side and care should be taken to avoid unnecessary exposure of the blood to the air. In either case, one-tenth of a cubic centimetre of a 50 per cent. solution of dextrose,

which has been sterilized at 100° C. on three successive days, for each 10 centimetres of blood to be taken, is placed in the defibrinating tube before the blood is drawn. Defibrination is effected by gently stirring or whipping with a glass rod or tube which extends through the cotton plug closing the tube. The whipping in of air, causing bubbles, must be avoided. The plug and rod may now be replaced by a plug from another tube of the same size.

"This defibrinated dextrose blood containing malarial plasmodia may be transferred to other tubes or incubated in the original tube. In any event the column of blood must be 2.5 to 5 cm. deep. This gives a column of serum 1.25 to 2.5 cm. deep above the cells and parasites, when the latter have settled. Supernatant serum more than 2.5 cm. deep has no advantage. When this is less than 1.25 cm. deep the parasites often die before segmentation occurs.

"The parasites live and develop at the top of the column of precipitated cells in a layer varying in thickness from 0.05 to 0.1 cm. . . . The parasites in the thin layer at the top of the column of cells develop and may be examined at any time by drawing a small quantity of cells from this layer by means of a capillary pipette. Some considerable practice is required to do this without drawing cells and dead parasites from just beneath this layer. . . . Great care should be taken in handling tubes containing cultures to keep them in an upright position. Tilting to the side results in burying and killing the living parasites in the thin layer at the top of the column of cells."

For cultivating more than one generation of parasites, they recommend the following method:

"The infected blood from the patient is centrifugalized sufficiently to force the leucocytes to the surface of the column of cells. . . . The supernatant fluid is drawn off and placed in culture tubes. The column of serum in each should be 1.25 to 2.5 cm. deep. Cells and plasmodia are carefully drawn from about the middle of the centrifugalized cells and planted at the bottom of the serum in the culture tubes. One to two tenths of a cubic centimetre of cells in a half-inch tube makes the thickest layer in which it is possible to get a homogeneous growth of parasites.

"Parasites in such leucocyte-free cultures develop, segment, and most of the merozoites enter new red blood-cells. These young parasites develop in the same manner as the first generation and sometimes reach the stage of segmentation. In fact, we have in one instance observed the development of three successive generations in such a culture. More often, however, the parasites begin to die out after the first segmentation and especially after the second. . . . In order to perpetuate the culture it is necessary to transfer a portion of the cells and parasites to a recently prepared tube containing fresh cells and serum. It is convenient to place the fresh serum in the culture tube and to take up in a large capillary pipette a portion of the cells and parasites of the culture and then about five times the amount of fresh cells. They are mixed in the pipette (avoid air) and then carefully spread on the bottom of the tube. The transplantation should be done within four or five hours of the time of maximum segmentation and therefore approximately every forty-eight hours for the tertian and æstivo-autumnal parasites."

Incubation of the cultures should be at 37° C., but the plasmodia will grow at 40 to 41° C., and 39° C. is a favorable temperature for cultivation.

Sinton has found that ascitic or hydrocele fluid is just as effective as blood serum in culturing malaria plasmodia and he adds to each 100 c.c. of such fluid 1.5 to 2.0 c.c. of a sterile 50 per cent. solution of dextrose.

Bass and Johns were able, in one instance, to secure five generations of plasmodia in cultures, but usually the parasites perish after the second or third generation. This fact has rendered the cultivation of the malaria



plasmodia of less practical importance than would otherwise be the case, as the technique is rather laborious for the results obtained.

### STAINING METHODS

Numerous staining methods have been devised for staining the PROTOZOA, but only those which have been found generally useful will be here considered.

**Staining Methods for Amœbæ.**—In staining either free-living or parasitic amœbæ, it is first necessary to fix the material to be stained, and for this purpose various methods of fixation have been recommended, including fixation with osmic acid, sublimate acetic acid mixture, and acetic acid solution of picric acid and corrosive sublimate. The most generally useful, and the one most used for the fixation of amœbæ, is Schaudinn's alcohol-corrosive sublimate mixture with acetic acid.

#### *Schaudinn's Solution of Alcohol and Corrosive Sublimate*

Saturated solution of corrosive sublimate ( $\text{HgCl}_2$ ) in distilled water....	2 parts
Absolute (or 96 per cent.) alcohol .....	1 part

These are mixed, and to each 100 c.c. is added 5 c.c. of glacial acetic acid. The mixture keeps indefinitely.

The staining methods which follow are all preceded by fixation of the material with this solution. An excellent method for staining amœbæ has already been described in the discussion of the diagnosis of the parasitic amœbæ, but the following methods may be substituted, if desired.

#### *Mann's Stain (Modified by Dobell)*

Aqueous solution of methyl blue, 1 per cent. ....	35 c.c.
Aqueous solution of eosin, 1 per cent. ....	45 c.c.
Distilled water .....	100 c.c.

The films of the material to be stained are fixed in the sublimate solution for from 10 to 20 minutes, washed in 50 per cent. alcohol, in 70 per cent. alcohol to which enough iodine has been added to give it a port wine color, in 80 per cent. alcohol, and finally in 90 per cent. alcohol, in each of which the films should be left for about 10 minutes. They are then placed in distilled water, in which they are allowed to remain for 10 minutes, and are then placed in the staining mixture and allowed to remain for from 4 to 12 hours, the exact period being determined by trial. After staining, the films are washed in distilled water and differentiated in 70 per cent. alcohol containing a little Orange G. (a few drops of a saturated solution added to 100 c.c. of 70 per cent. alcohol). When differentiated properly, and the differentiation has to be controlled by microscopic examination, the films are washed in distilled water, 30, 50, 70, and 90 per cent. alcohol, leaving them in each for a period of at least 5 minutes, and are finally placed in absolute alcohol and allowed to remain

for 10 minutes. They are then transferred to equal parts of alcohol and xylol, and finally cleared in pure xylol, and mounted in xylol balsam. The films should remain in the mixture of xylol and alcohol for 5 minutes, and the xylol then added, when clearing occurs at once. *At no time in the staining process should the films be allowed to dry.*

*Rosenbusch Stain.* Two solutions are used in staining by this method.

Solution 1. A 1 per cent. solution of hæmatoxylin in 95 per cent. alcohol. (Must be at least 10 days old when used.)

Solution 2. A saturated solution of lithium carbonate in distilled water.

The two solutions are mixed for staining by adding 5 or 6 drops of the lithium carbonate solution to each 10 c.c. of the hæmatoxylin solution.

The method of using this stain is as follows :

1. Fix the films in Schaudinn's alcohol-sublimate solution for 10 minutes.

2. Place films for five minutes in 70 per cent. alcohol; 70 per cent. alcohol plus enough iodine to give it a port wine color; 90 per cent. alcohol; and distilled water.

3. Place films in a 3.5 per cent. solution of iron-alum in distilled water and allow them to remain for one-half hour or longer.

4. Wash thoroughly in distilled water.

5. Stain with the hæmatoxylin-lithium carbonate mixture for from 5 to 20 minutes.

6. Wash thoroughly in distilled water.

7. Differentiate with a weak iron-alum solution. The solution used in Step 3 diluted with 3 parts of distilled water is recommended.

8. Wash thoroughly in distilled water; 95 per cent. alcohol; absolute alcohol, leaving in each at least 10 minutes.

9. Clear with xylol and mount in xylol balsam.

*At no time during the fixing and staining process should the films be allowed to dry or they will be valueless.*

#### *Walker's Stain*

Hæmatoxylin crystals .....	1 gm.
Saturated aqueous solution of ammonia alum .....	100 c.c.
Distilled water .....	300 c.c.
Thymol .....	a crystal

The hæmatoxylin crystals are dissolved in the water by the aid of heat and the other substances added. The stain should ripen for 10 days in a flask loosely stoppered with cotton, and after ripening it should be kept in the dark.

The method of using this stain is as follows :

The films are fixed in Schaudinn's alcohol-sublimate solution for 10 to 15 minutes, washed in distilled water, stained in the aqueous alum-hæmatoxylin from 3 to 5 minutes, passed through 50, 60, 70, 90, and 95 per cent. alcohol into absolute alcohol, leaving in each at least 5 minutes, cleared in xylol, and mounted in xylol balsam.

**Staining Methods for Flagellates and Plasmodia.**—If it is desired to obtain preparations showing the minute structural details of the nuclei of flagellates, as the intestinal flagellates and trypanosomes, one of the methods recommended for the staining of amœbæ should be used, preceded by wet-fixation with Schaudinn's sublimate-alcohol solution. In using these methods the same precaution should be observed regarding the drying of the films during the process of fixation and staining, for if the films dry at any stage of the process the results will be worthless. In staining intestinal flagellates or trypanosomes with any of the methods used for amœbæ, the same steps are followed as if staining the amœbæ and just as excellent results will be obtained.

However, in clinical practice, it is seldom necessary to stain the intestinal flagellates and for the staining of trypanosomes, leishmania, and the malaria plasmodia, one of the modifications of the Romanowsky stain is now universally employed. With any of the modifications of this stain fixation and staining is accomplished at the same time, the alcohol in the staining solution acting as the fixative.

In my own work I have found Wright's modification of the Romanowsky stain most excellent and I prefer it to any other for routine purposes. It is an excellent stain for trypanosomes, leishmania, and the malaria plasmodia, and is also useful in staining the intestinal flagellates.

*Wright's Stain.* The method of preparing and using this stain is as follows:

In a flask containing 100 c.c. of distilled water, add 0.5 gm. of sodium bicarbonate, dissolve, and then slowly add, while shaking, 1 gram of methylene blue; heat for one hour in a steam sterilizer after the steam is up, and then cool the solution. A considerable amount of methylene blue will remain undissolved, but this should be allowed to remain in the solution.

Make a solution of yellow aqueous eosin by adding 1 gram of the eosin to 1,000 c.c. of distilled water. Add this solution slowly, while stirring, to the cooled methylene blue solution, which has been poured into a white dish or bowl. The eosin solution is added until a well-marked precipitate appears and the surface of the mixture is covered with a greenish metallic scum. Test repeatedly, while adding the eosin solution, by placing a drop of the mixture upon a piece of filter paper. When sufficient eosin has been added, a well-marked pink halo should surround

the small amount of precipitate upon the paper. When this occurs, allow the mixture to stand for 15 minutes, and then filter the entire amount through one small filter paper; the precipitate is saved, dried in a hot-air oven at 60° C., and the greenish powder so obtained is used in preparing the staining solution.

The *staining solution* is prepared by taking 0.3 gram of the powdered precipitate and adding it to 100 c.c. of pure methylic alcohol (Merck's Reagent Alcohol). Filter, and add enough of the alcohol to bring up the entire amount to the original 100 c.c. The staining solution is now ready for use. It should be stored in the dark and will keep for weeks.

The *method of using the stain* is as follows: Add a few drops of the staining solution to the preparation to be stained and let stand for 3 to 5 minutes. This fixes the preparation. Then add enough distilled water to cause a greenish metallic scum to appear upon the surface of the solution; let stand for from 5 to 20 minutes, according to the material to be stained, wash in running distilled water, and examine directly when dry. The exact time for staining the various protozoan organisms with this stain is given in the discussion of the diagnosis of the organisms in the body of the text.

The final washing with distilled water is very important as it rids the specimen of the precipitate that is formed during staining and also aids in differentiating the staining of the cytoplasm of cells and the chromatin of the nuclei of the PROTOZOA. Care should be taken that enough stain is added in the beginning so that evaporation will not occur before the distilled water is added, as otherwise the specimens will be ruined.

The staining reactions with this stain are given in the discussion of the various organisms which are well stained by this method.

*Leishman's Stain.* This is an excellent stain for leishmania, trypanosomes, and the malaria plasmodia. It is prepared as follows:

1. Make a 1 per cent. solution of medicinal methylene blue in distilled water made alkaline by the addition of 0.5 per cent. of sodium carbonate. Heat for 12 hours at 65° C. in an oven and allow to stand for 10 days at room temperature before using.
2. Prepare a 1 to 1,000 solution of aqueous eosin.
3. Equal volumes of these two solutions are mixed in an open dish and allowed to stand for 6 to 12 hours, during which time the mixture should be stirred at intervals.
4. Collect the precipitate that results upon one small filter paper and wash with distilled water until the washings are colorless or a pale blue; the precipitate is then dried and powdered.
5. To make the staining solution, dissolve 0.15 gm. of the powder in 1,000 c.c. of Merck's methylic alcohol. (Reagent.)



The method of using this stain is the same as with Wright's stain, but I have not found it as generally satisfactory as the latter stain.

*Giemsa's Stain.* This is a very valuable stain for trypanosomes, leishmania, and malaria plasmodia, but it is apt to overstain unless carefully handled. It can be bought, and very good preparations are on the market, and as it is a difficult stain to prepare, it is recommended that a reliable commercial Giemsa be purchased if it is to be used. Owing to the difficulty of preparation and the fact that it easily overstains, I have always preferred the Wright stain in routine diagnostic work. The formula of the stain is as follows:

Azur II-eosin .....	3 gm.
Azur II .....	0.8 gm.
Glycerin (Merck pure) .....	250 c.c.
Methyl alcohol C. P. ....	250 c.c.

Dissolve the Azur II-eosin and Azur II, with constant shaking, in the glycerin at a temperature of 60° C. After solution is complete, add the alcohol, which has been previously heated to 60° C. This mixture is shaken well, allowed to stand at room temperature for 24 hours, and then filtered into a chemically clean, sterilized air-tight stock bottle. During the filtration the funnel must be covered with an inverted watch-glass to protect the hygroscopic fluid from moisture. Store in the dark.

The method of using the stain is as follows:

The films to be stained are fixed in absolute alcohol for 10 minutes and then stained with the staining solution, which has been diluted with distilled water in the proportion of 1 drop of the stock-staining solution to 1 c.c. of water. It is well to make up from 10 to 25 c.c. of the diluted stain at a time, and to add to it a 1 per cent. potassium carbonate solution in distilled water in the proportion of 1 drop to each 10 c.c. of the diluted stain.

The length of time of staining with Giemsa varies with the organism to be stained, but for trypanosomes, leishmania, and the malaria plasmodia the stain should be allowed to act for from 5 to 15 minutes. After staining, the films should be thoroughly washed in running distilled water, dried, and examined without a cover-glass, or, if a cover-glass is used, the preparations should be mounted in xylol balsam.

The staining reactions of cytoplasm, nucleus, etc., are the same with this stain as with the Wright or Leishman stain.

*Jenner's Stain.* This is a very good stain for trypanosomes, leishmania, and the malaria plasmodia, and is prepared as follows:

Equal parts of a 1.2 per cent. solution of water-soluble eosin in distilled water, and a 1 per cent. aqueous solution of medicinal methylene blue, are mixed together and allowed to stand at room temperature for 24 hours. At the end of this time a coarse precipitate has formed of

dark color, with a metallic lustre. This is collected by filtration through a small filter paper and washed in distilled water until the filtrate is almost colorless. The precipitate is then collected in an air-tight bottle and stored in the dark.

The staining fluid is prepared by adding 0.5 gm. of the precipitate to 100 c.c. of pure methyl alcohol, and the blood-smears, or other material, are stained in the same manner as with the Wright stain.

*Ross Thick Film Method.* This method is useful in staining blood in suspected cases of malaria in which the ordinary blood examination is negative, owing to the small number of plasmodia present. It has also been used in malaria surveys by some observers.

A large drop of blood from the patient is collected upon a microscopic slide at, or near, the middle, and with a needle is spread until it covers an area of about one-half inch in diameter. The smearing may be done with a platinum loop, and the film should be made as evenly as possible. The preparation is now placed aside and allowed to dry. Drying may be hastened by placing the preparation in the incubator at 37° C.

After the blood-smear is dry, the slide is placed in a mixture composed of 50 c.c. of commercial ethyl alcohol containing 10 drops of chemically pure hydrochloric acid, and removed when the hæmoglobin is completely dissolved, a process which is usually complete within from 10 minutes to half an hour, depending upon the amount of blood to be de hæmoglobinized.

The preparation is now washed in running water for from 10 minutes to half an hour to remove the acid, for unless this is removed staining is practically prevented.

After washing the preparation is stained with Wright's stain or one of the other stains described and then washed in distilled water, dried, and examined without a cover-glass.

In specimens stained in this manner it should be remembered that the erythrocytes are not distinctly stained and that the malaria plasmodia appear as though free in the blood plasma.

*Staining of Old Blood-films.* If blood-films are several weeks old they will not stain well with any of the modifications of the Romanowsky stain, as the Wright or Leishman stains, and valuable material is frequently rendered useless because of this phenomenon. Such blood-films may be stained well if treated in the following way, as recommended by Daniels:

Before staining, the slide containing the blood-film is placed in a mixture of absolute alcohol and glacial acetic acid, 30 c.c. of alcohol containing 3-5 drops of glacial acetic acid. The slides are left in this for five or ten minutes and then washed in distilled water, after which they are ready to be stained.

If blood-smears have been sent to a laboratory from a distance and several weeks have elapsed since the films were made, it is always well to treat them in the manner recommended by Daniels before attempting to stain them with the Wright or other modification of the Romanowsky stain. Blood-smears exposed to the heat of a tropical climate for even a few days may stain poorly and this method will often greatly improve their staining qualities.

*Normal Salt Solution and Distilled Water.* Both normal salt solution and distilled water are commonly used in diluting the fæces during examination for amœbæ, flagellates and ciliates, and it should be remembered that both should be freshly prepared or examined microscopically before use, in order to avoid contaminating specimens with coprozoic organisms. Coprozoic amœbæ, flagellates and ciliates often occur in normal salt solution and distilled water that are not freshly prepared and this fact should be remembered in using these materials.





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